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Significance of molecular phylogenetic analyses for paleobotanical investigations on the origin of angiosperms

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ABSTRACT

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Molecular phylogenetic analyses have provided increasing evidence that angiosperms are not related to Gnetales, thus contradicting the anthophyte hypothesis based on morphological cladistic analyses and throwing the question of angiosperm relatives back to paleobotanists. Previous analyses of gene sequences based on a molecular clock conflicted with the fossil record in indicating a Late Paleozoic or Triassic origin of the angiosperms, but closer examination suggests that these dates were biased by the use of herbaceous taxa with accelerated rates of molecular evolution. Despite uncertainty on angiosperm relatives, analyses of many genes consistently place *Amborella*, Nymphaeales, *Austrobaileya*, *Trimenia* and Illiciales (the 'ANITA grade') at the base of extant angiosperms, possibly followed by Chloranthaceae. Molecular phylogenies imply that the first crown-group angiosperms had columellar exine structure, suggesting that Hauterivian-Barremian reticulate-columellar monosulcates may be closer to the origin of angiosperms than was thought when granular Magnoliales were believed to be basal. Hauterivian pollen with a verrucate tectum and microspinules is especially similar to *Amborella*. The ANITA lines and Chloranthaceae have ascidiate carpels sealed by secretion and often exotestal seeds, fitting the abundance of such carpels and seeds in Barremian-Aptian mesofloras. Similarities between Aptian angiosperm leaves and ANITA taxa, such as chloranthoid teeth and variable stomatal structure, also suggest that Early Cretaceous angiosperms were more primitive than previously appreciated. Molecular results may help refine search images for extinct angiosperm relatives, away from Gnetales and toward groups such as *Caytonia*, glossopterids, Bennettitales and corystosperms. Since molecular data place the vesselless taxa *Amborella* and Nymphaeales at the base of the angiosperms, the presence of vessels is not evidence that gigantopterids are related to angiosperms. The conclusion that columellar structure is ancestral reaffirms the potential of Triassic reticulate-columellar Crinopolles pollen as angiosperm relatives.

Key-words—Angiosperms, Paleobotany, Cretaceous, Phylogeny, Molecular systematics.

आवृतबीजियों के उद्गम हेतु पुरावानस्पतिक अन्वेषणों में आणविक जातिवृत्तीय विश्लेषणों का महत्व

जेम्स ए. डॉयल

सारांश

आणविक जातिवृत्तीय विश्लेषणों से यह प्रमाणित हुआ है कि आवृतबीजी नीटेलीज़ से सम्बन्धित नहीं हैं। इससे संरचनात्मक क्लैडिस्टिक विश्लेषणों पर आधारित एन्थोफ़ाइट अवधारणा का खण्डन होता है तथा पुरावनस्पतिवैज्ञानिकों के समक्ष आवृतबीजियों के सम्बन्ध में अनेक प्रश्न मुँह उठाए खड़े हो जाते हैं। आणविक घड़ी के आधार पर जीन अनुक्रमों

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के अश्मित आंकड़ों के साथ किए गए विगत विश्लेषण, जो आवृतबीजियों हेतु अन्तिम पेलियोज़ोइक अथवा ट्रायसिक उद्गम का संकेत करते थे, विरोधाभासी हैं, किन्तु गहन परीक्षण द्वारा प्रस्तावित किया जाता है कि ये काल निर्धारण आणविक विकास की त्वरित दर के साथ शाकीय वर्गकों के प्रयोग के कारण एकांगी हो गए। आवृतबीजियों के साहचर्यों की अनिश्चितता के बावजूद विद्यमान आवृतबीजियों के आधार पर लगातार *एम्बोरेला, निम्फ़ीलीज़, ऑस्ट्रोबेलीया, ट्राइमीनिया* तथा एल्लीसिएलीज़ (ANITA ग्रेड) लगातार अवस्थित पाए गए, जिसके पश्चात क्लोरैन्थेसी उपस्थित है। आणविक जातिवृत्त इंगित करता है कि प्रथम शिखर समूह के आवृतबीजियों की संरचना स्तम्भीय एक्ज़ाइन की भाँति की थी, इससे प्रस्तावित होता है कि हाउटेरेवियन-बैरीमियन जालिकामय-स्तम्भीय एकसकोषी आवृतबीजियों के उद्गम से पूर्व में सोची गई दूरी की अपेक्षा अधिक निकट हो सकते हैं, जब कणिकामय मैग्नोलिएलीज़ आधारीय समझे जाते थे। कणिकामय टेक्टम तथा सूक्ष्म शूलिकाओं से युक्त हाउटेरेवियन परागकण विशेषकर *एम्बोरेला* के समरूप हैं ANITA रेखाएँ तथा क्लोरैन्थेसी उत्सर्जन द्वारा तथा प्रायः एक्सोटेस्टल बीजों द्वारा बन्द की हुई हैं, जो बैरीमियन-एप्टियन मीज़ो वनस्पतिजातों में ऐसे अण्डपों तथा बीजों की प्रचुरता की उपयुक्तता सिद्ध करती है। एप्टियन आवृतबीजी पत्तियों तथा ANITA वर्गकों में क्लोरैन्थॉयड दन्तों तथा परिवर्ती रंध्रीय संरचना जैसी समरूपताएँ प्रस्तावित करती हैं कि पूर्व में समीक्षित किए गए आवृतबीजियों की अपेक्षा प्रारंभिक क्रिटेशस आवृतबीजी कहीं अधिक अपरिष्कृत थे। आणविक परिणाम नेटेलीज़ तथा संगत समूहों, जैसे—*केटोनिया,* ग्लॉसोप्टेरिड्स, बेनीटाइटेलीज़ तथा कोराइस्टोस्थर्मों से विलग विद्यमान आवृतबीजी सम्बन्धियों हेतु परिष्कृत अनुसन्धान प्रतिरूप निर्मित करने में सहायक हैं। चूँकि आणविक आंकड़े वाहिकारहित वर्गकों *एम्बोरेला* तथा निम्फ़ीलीज़ को आवृतबीजियों की तलहटी में रखते हैं, अतः वाहिकाओं की उपस्थिति से प्रमाणित नहीं होता कि जाइगैप्टोप्टेरिड्स आवृतबीजियों से सम्बन्धित हैं इससे यह निष्कर्ष निकलता है कि स्तम्भीय संरचना अपेक्षाकृत प्राचीन है। इससे ट्रायसिक जालिकामय-स्तम्भीय क्राइनोपोलीज़ परागकणों के आवृतबीजियों के सम्बन्धी होने के प्रबल प्रमाण पुष्ट होते हैं।

संकेत शब्द—आवृतबीजी, पुरावनस्पतिविज्ञान, क्रिटेशस, जातिवृत्त विज्ञान, आणविक तंत्र विज्ञान.

INTRODUCTION

Over the past 40 years, paleobotanical studies of Cretaceous fossils, first pollen and leaves, more recently flowers, fruits and seeds in the 'mesofossil' record have provided many indications on the course of early angiosperm evolution, for example supporting the view that 'magnoliids' include the most primitive living angiosperms and 'Amentiferae' are advanced (Crane *et al.*, 1995; Doyle, 1969, 1978; Doyle & Hickey, 1976; Friis & Crepet, 1987; Friis *et al.*, 2000; Hickey & Doyle, 1977; Muller, 1970; Upchurch, 1984; Wolfe *et al.*, 1975). These studies have provided no direct evidence on links between angiosperms and other seed plants, but beginning in the 1980s cladistic analyses of morphological data from living and fossil seed plants appeared to narrow the range of viable hypotheses on this problem, indicating that seed plants and angiosperms are both monophyletic groups and focusing attention on Gnetales, Bennettitales, glossopterids, *Caytonia* and other 'Mesozoic seed ferns' as possible angiosperm relatives (Crane, 1985; Doyle & Donoghue, 1986; Loconte & Stevenson, 1990; Nixon *et al.*, 1994; Rothwell & Serbet, 1994; Doyle, 1996, 1998b).

In the past 10 years, cladistic analyses of molecular data, of necessity restricted to living plants, have provided a vast and completely independent body of evidence on these questions. Although these studies cannot directly address the relationships of fossil taxa to the angiosperms, they do bear on competing hypotheses when these make different predictions on relationships among living taxa. As an observer of both fields, I have been struck not only by conflicts between the two lines of evidence, which have perhaps attracted more attention (Axsmith *et al.*, 1998; Doyle, 1998a; Goremykin *et al.*, 1996), but also by unexpected agreements, and by ways in which insights from one field may suggest new directions for research in the other. This paper will explore both the conflicts and agreements, considering four closely interrelated questions: what the angiosperms came from, when they originated, what the first angiosperms were like, and how answers to the last question may shed light on the first. I will discuss these questions in a cladistic framework, which allows us to generate and test phylogenetic hypotheses in a coherent, explicit fashion.

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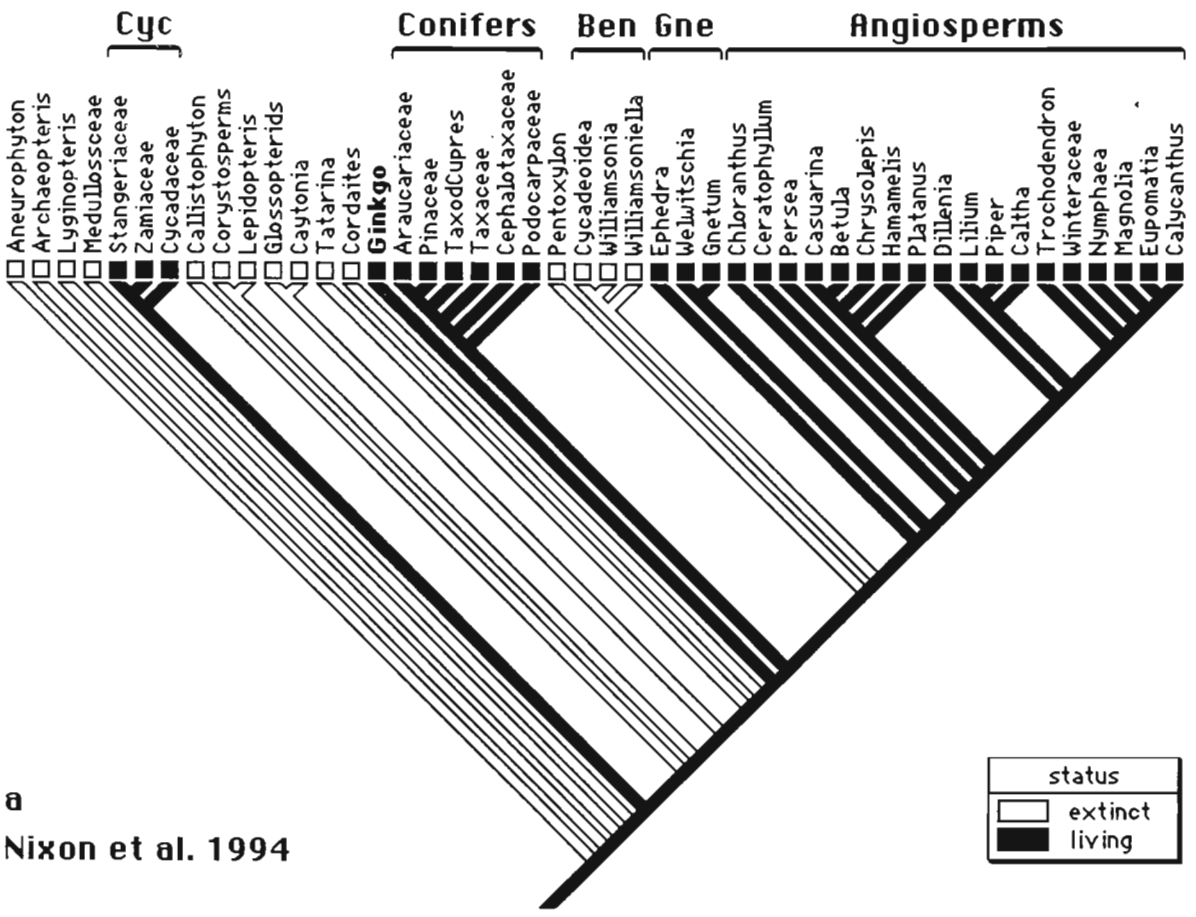
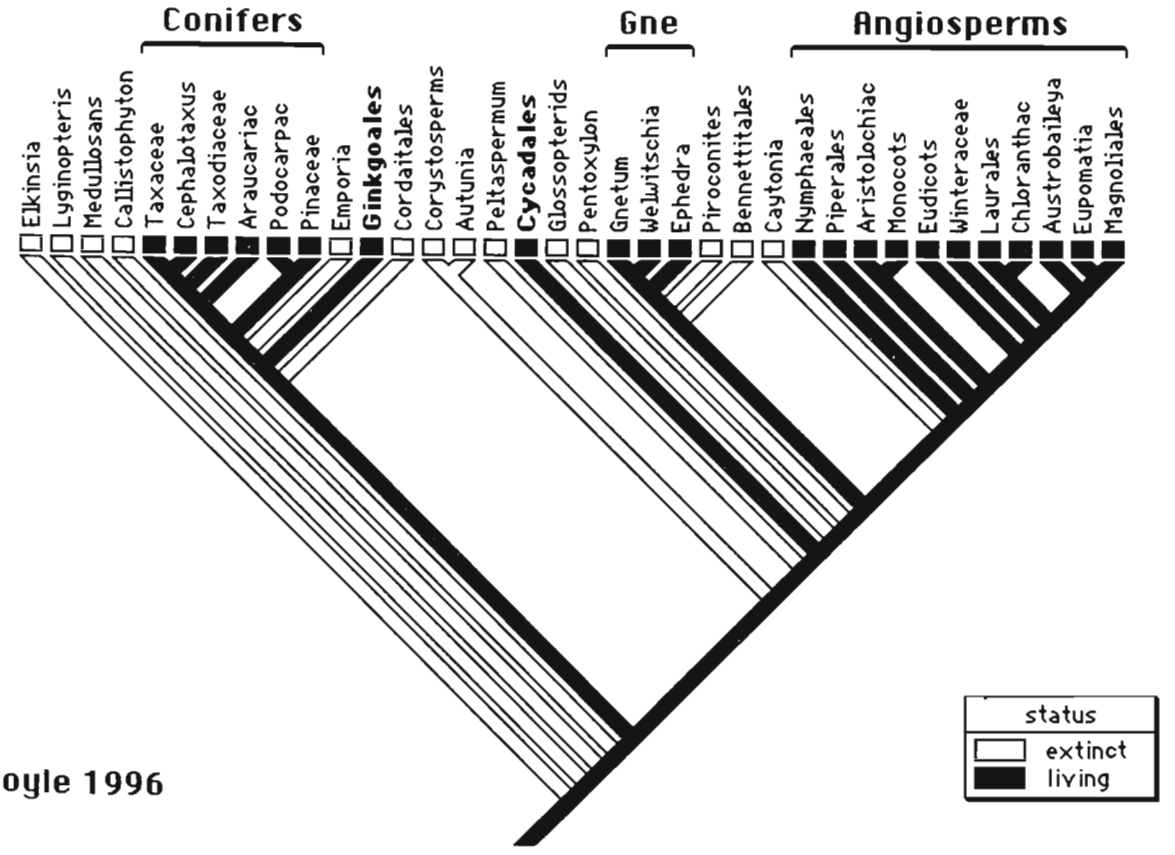
Fig. 1—Representative most parsimonious trees from morphological cladistic analyses of seed plants; (a) Nixon *et al.* (1994), (b) Doyle (1996). Extant lines are indicated in black, extinct lines in white.



a

Nixon et al. 1994



b

Doyle 1996

SEED PLANT PHYLOGENY AND THE ORIGIN OF ANGIOSPERMS

The first question, what the angiosperms came from, can be addressed cladistically by asking how angiosperms are related to other seed plants - what are their closest outgroups - and examining character states shared by these outgroups. This is a case in which most molecular data conflict with morphological cladistic analyses of living and fossil seed plants. Whereas before the application of cladistics there was little agreement on this topic, cladistic analyses all associated angiosperms with Mesozoic Bennettitales and living Gnetales, in a clade called the anthophytes, although they did disagree on just how these groups were related and what other taxa they were related to. Previously, it was widely thought that Gnetales had nothing to do with angiosperms and were instead related to conifers and other coniferophytes (Bailey, 1949; Doyle, 1978; Eames, 1952). The first major analysis, by Crane (1985), identified Gnetales as the sister group of angiosperms, Bennettitales and *Pentoxylon* as the second outgroup, and corystosperms, *Caytonia* and glossopterids as outgroups of the anthophytes. The trees of Doyle and Donoghue (1986) differed in placing angiosperms at the base of the anthophytes, somewhat further from Gnetales. Two of the most divergent results are shown in Fig. 1. In trees of Nixon *et al.* (1994), anthophytes were related to conifers rather than to Mesozoic seed ferns and angiosperms were actually nested within Gnetales (Fig. 1a). In my own latest analysis (Doyle, 1996), *Caytonia* was directly linked with angiosperms, but Gnetales were still their closest living relatives (Fig. 1b). As discussed in Doyle (1994), trees of these different types have very different implications for origin of the angiosperm bitegmic ovule and the carpel. Those that associate anthophytes with *Caytonia* or glossopterids suggest that the angiosperm outer integument is derived from a cupule, whereas those that associate anthophytes with conifers suggest it is homologous with the perianth of Gnetales and derived from scale leaves on an axillary fertile short shoot of the type seen in cordaites and early conifers.

Although molecular analyses cannot shed light on the relationships of angiosperms to Bennettitales, *Caytonia* and other fossils, they can address the relationship between angiosperms and Gnetales: are these groups related at all, and if so, are they monophyletic sister groups, or are angiosperms nested within Gnetales? Early molecular analyses indicated that angiosperms and Gnetales are both monophyletic (Fig. 2), with strong statistical support as measured by bootstrap analysis (Felsenstein, 1985), refuting the view that angiosperms are nested in Gnetales. However, they gave inconsistent results on relationships of the two groups. Some analyses of rDNA indicated that they are sister groups (Hamby & Zimmer, 1992; Stefanovic *et al.*, 1998; Fig. 2a), but this result was weakly supported. Other analyses of rDNA (Hamby & Zimmer, 1992) and the chloroplast gene *rbcL* (Albert *et al.*, 1994) placed Gnetales at the base of seed plants, with angiosperms linked with cycads, *Ginkgo* and conifers (Fig. 2b), or else reversed Gnetales and angiosperms (Hasebe *et al.*, 1992; Fig. 2c). These variations are a function of rooting - where outgroups attach to the seed plant tree; otherwise, the three trees are the same. There is reason to expect that the rooting of seed plants should be difficult: the conifer, cycad and ginkgo lines extend back to the middle Late Carboniferous or the Permian and presumably split not long before and there has been a long time since then for convergences and reversals on the lines leading to living seed plants, resulting in so-called long-branch attraction (Donoghue & Sanderson, 1992; Doyle, 1998a; Felsenstein, 1978). However, in trees first seen in analyses of chloroplast ITS sequences (Goremykin *et al.*, 1996) and 18S rDNA (Chaw *et al.*, 1997), angiosperms are basal in seed plants and Gnetales are linked with conifers (Fig. 2d). With this type of tree, there is no way to reroot seed plants such that angiosperms and Gnetales are related.

In all these studies, bootstrap support for relationships among seed plants was relatively low, so it seemed possible to argue that the morphological evidence for the anthophyte hypothesis could still be accepted (Doyle, 1998a). However, this situation has changed since 1998: many studies based both on single genes and on several genes combined have indicated that Gnetales are more closely related to conifers than to angiosperms, with much higher bootstrap support (Bowe *et al.*, 2000; Chaw *et al.*, 2000; Frohlich & Parker, 2000; Hansen *et al.*, 1999; Qiu *et al.*, 1999; Samigullin *et al.*, 1999; Winter *et al.*, 1999). In fact, the multigene analyses of Qiu *et al.* (1999), Bowe *et al.* (2000) and Chaw *et al.* (2000) actually nested Gnetales within conifers, linked with Pinaceae (Fig. 2e), with most critical nodes supported by bootstrap values of 90-100%. The main variation is that 18S rDNA alone indicates fairly strongly that Gnetales are the sister group of conifers rather than nested within them (Bowe *et al.*, 2000; Chaw *et al.*, 1997), as does the fact that conifers are united by loss of one copy of the large inverted repeat in the chloroplast genome, whereas Gnetales retain both copies (Raubeson & Jansen, 1992). In any case, all these studies are unequivocal in rejecting a relationship between Gnetales and angiosperms (Donoghue & Doyle, 2000). These results are consistent with morphological similarities between Gnetales and conifers cited in pre-cladistic studies, such as linear leaves, lack of scalariform pitting in the primary xylem, circular-bordered pits with tori in the secondary xylem, and compound strobili made up of axillary fertile short shoots (Bailey, 1949; Carlquist, 1996; Doyle, 1978; Eames, 1952), which were outnumbered by anthophyte similarities in morphological cladistic analyses.

These results are not definitive, since other recent studies have produced trees in which Gnetales are the sister group of other living seed plants (Fig. 2b). Sanderson *et al.* (2000) found trees of this sort in parsimony analyses of the chloroplast genes

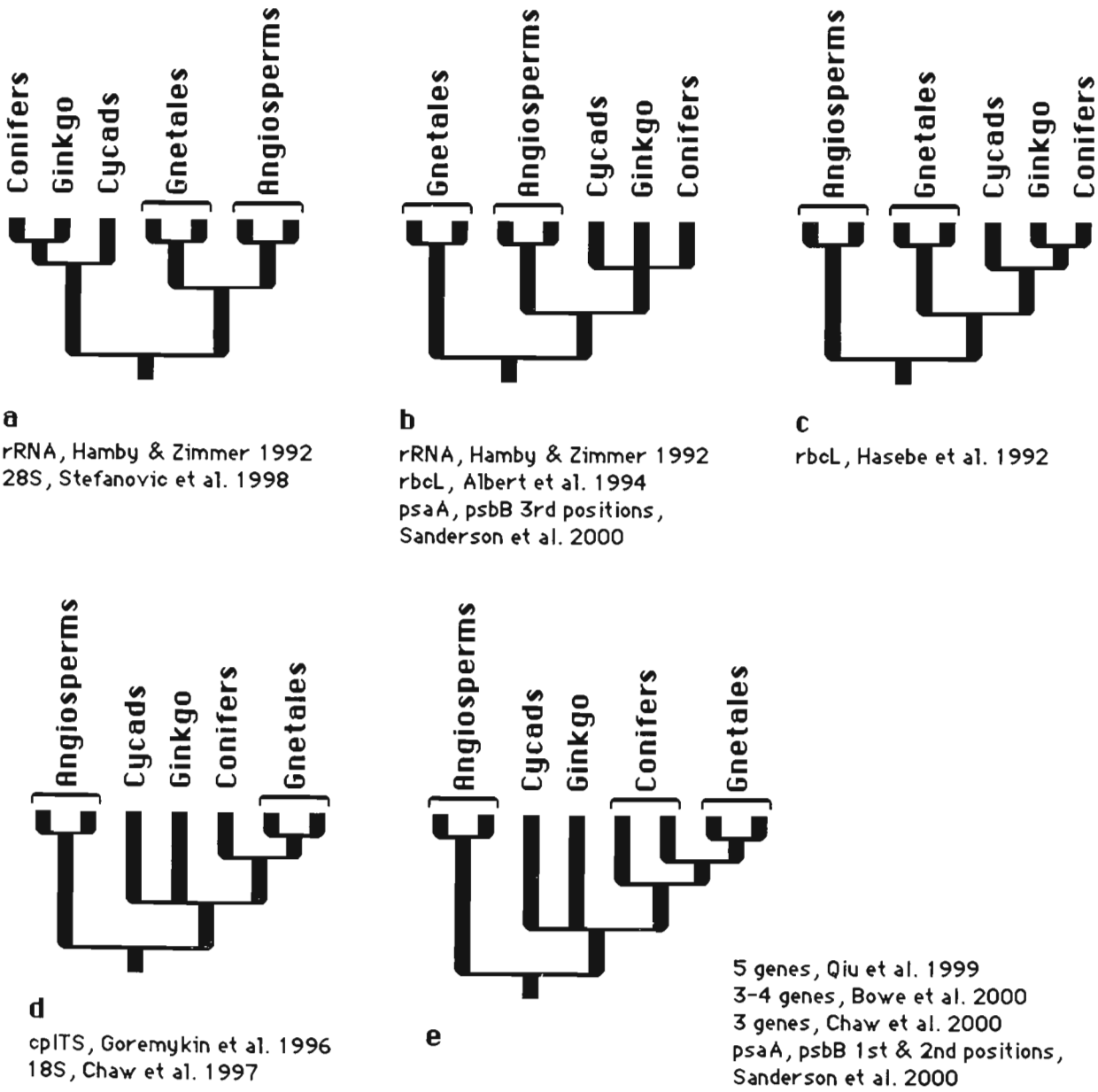


Fig. 2—Seed plant relationships found in analyses of molecular data.

psaA and *psbB*. However, they obtained divergent results when they analyzed different nucleotide positions in each codon: Gnetales nested in conifers based on first and second codon positions, but Gnetales basal in seed plants based on third positions. Since third positions evolve more rapidly, there is reason to suspect that the latter result may be due to long-branch attraction. Consistent with this view, Sanderson *et al.* found Gnetales nested in conifers when they analyzed third positions of *psaA* with maximum likelihood, which is believed to counteract long-branch attraction. Combined analyses of 17 chloroplast genes (Rai *et al.*, 2001) also gave trees with Gnetales basal, but again some subsets of the data and methods of analysis placed Gnetales in conifers. Rydin *et al.* (2002) found trees with Gnetales basal when they analyzed *rbcL* and

atpB (another chloroplast gene) and these genes combined with 18S and 26S rDNA. However, analysis of *rbcL* and *atpB* with exclusion of nucleotide transitions (which are more common than transversions and therefore more likely to cause long-branch attraction) gave trees with Gnetales nested in conifers. Combined analyses of 18S and 26S linked angiosperms with Gnetales (like some earlier rDNA analyses), but with low support.

Despite these uncertainties, the continued failure of molecular data to support the anthophyte hypothesis suggests that paleobotanists should begin to consider the implications of alternative trees. Trees that link Gnetales with conifers may deserve more attention than those with Gnetales basal, since they are more consistent with the conifer-like morphological features of Gnetales and harder to ascribe to long-branch attraction.

Most molecular studies that associate Gnetales with conifers are disconcerting in indicating that living gymnosperms are monophyletic, as the sister group of angiosperms (although this result is not strongly supported - trees with cycads basal or on the line to angiosperms are often almost as parsimonious). Since conifers extend back to the Late Carboniferous, this implies that the line leading to angiosperms goes back this far too – an apparent conflict with the stratigraphic record (Axsmith *et al.*, 1998; Doyle, 1998a). However, this result does not mean that angiosperms and gymnosperms evolved separately from progymnosperms, or that angiosperms originated in the Paleozoic. Since these trees include only living taxa, there could be any number of early seed ferns attached below the split of angiosperms from other living groups, and any number of Permian and Mesozoic fossils attached to the stem lineage leading to angiosperms. This point is illustrated by the tree in Fig. 3a, obtained by analyzing the data set of Doyle (1996) with living gymnosperms constrained to form a clade and Gnetales forced into conifers, and allowing fossils, shown in white, to attach wherever is most parsimonious. The Jurassic fossil *Piroconites*, previously linked with Gnetales, was removed because TEM studies indicate that its supposedly ephedroid exine structure was misinterpreted (Osborn, 2000); this had no effect on unconstrained trees. Late Devonian and Carboniferous seed ferns (*Elkinsia* through *Callistophyton*) still diverge at the base of the tree, and glossopterids, *Pentoxylon*, Bennettitales (in some trees) and *Caytonia* are still associated with angiosperms. Fig. 3b shows a tree from a similarly constrained analysis of the data set of Nixon *et al.* (1994); glossopterids and *Caytonia* are not associated with angiosperms, but Bennettitales are.

Whereas the anthophyte hypothesis suggested that studies of living Gnetales might shed light on the origin of angiosperm features such as double fertilization and endosperm (Friedman, 1992, 1994), molecular results imply that any progress in reconstructing the origin of angiosperms must come from paleobotany. Unfortunately, this task now looks more difficult than it formerly did, since morphological data and methods appear to have given dramatically incorrect results in the case of Gnetales. Before new analyses can be undertaken, we need a complete reappraisal of methods, particularly the way we analyze morphological characters, to understand why previous analyses went so wrong, and whether this could have been avoided. Several characters that seemed to associate angiosperms and Gnetales differ in detail in the two taxa (Donoghue & Doyle, 2000). For example, the tunica in the apical meristem consists of two cell layers in angiosperms but only one in Gnetales; the megaspore wall is thin in Gnetales but completely absent in angiosperms; and double fertilization in Gnetales produces two zygotes, not a zygote and a triploid endosperm nucleus (see references in Doyle, 1996; Doyle & Donoghue, 1986). Another character, granular exine structure, is discussed below. Although in all these cases the gnetalian state could be ancestral to the angiosperm one, the differences could equally well be evidence of convergence. We may also need new data on Mesozoic fossils - either new taxa or new data on characters of known taxa, such as seed cuticles (stressed by Harris, 1954 but generally neglected since then) and stem anatomy. We should also face the possibility that the number of morphological character states in seed plants is too small and the probability for homoplasy during their long evolutionary history too high for reliable cladistic inference ('character state exhaustion': Wagner, 2000), perhaps requiring discovery of new fossils and/or integration of stratigraphic data into phylogenetic analyses. However, molecular data do give indirect indications about groups that need more attention in the search for angiosperm relatives: not Gnetales and related fossils, but rather Bennettitales, *Caytonia*, glossopterids, corystosperms, and more poorly known 'seed ferns' in Triassic and Jurassic floras (cf. Anderson & Anderson, 1997).

AGE OF THE ANGIOSPERMS

The second question, when the angiosperms originated, is another case in which fossil and molecular data appear to conflict. Here it is important to distinguish two ages (Doyle & Donoghue, 1993): the time when the stem lineage leading to

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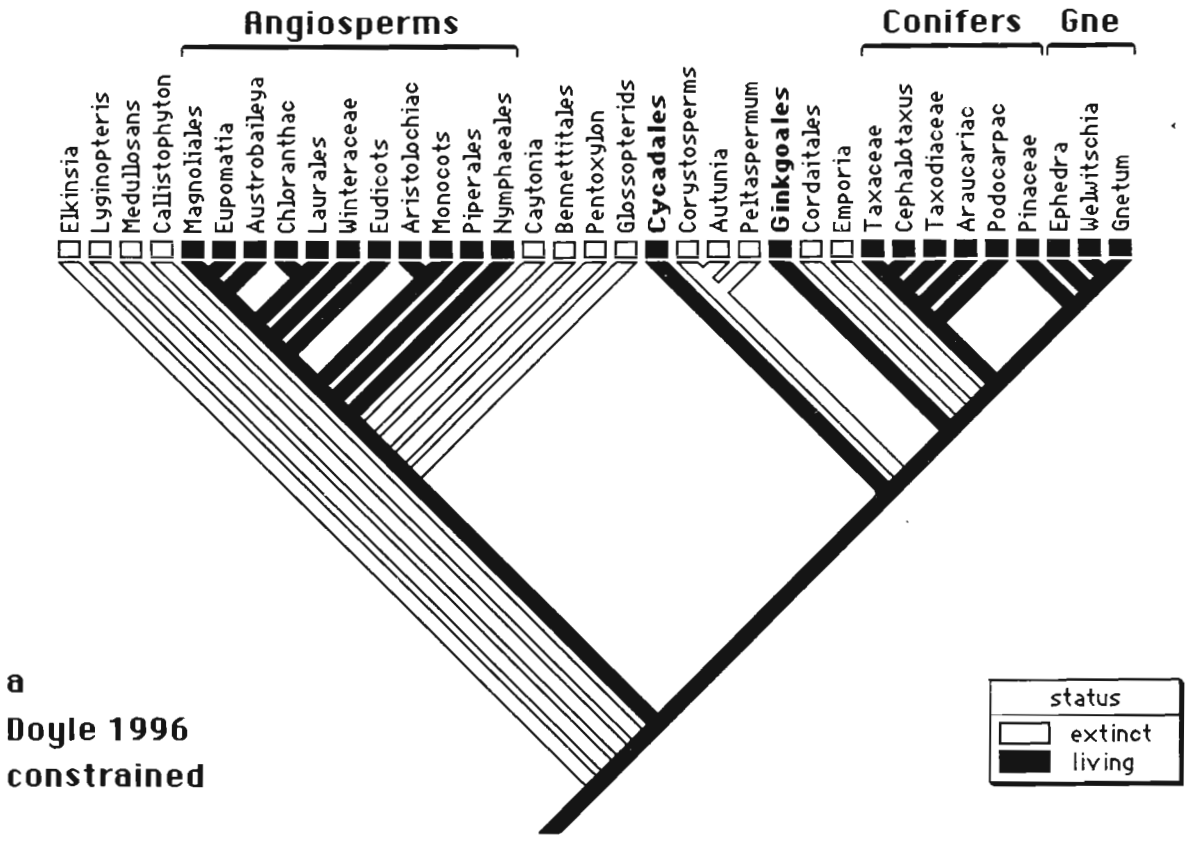
Fig. 3—Representative trees based on the data sets of (a) Doyle (1996) and (b) Nixon *et al.* (1994), with extant gymnosperms and angiosperms constrained into a sister group relationship and Gnetales forced into conifers with Pinaceae, as in recent molecular analyses (Fig. 2e). Extant lines are indicated in black, extinct lines in white.


Angiosperms
Conifers
Gne
Elkinsia
Lyginopteris
Medullosans
Callistophyton
Magnoliales
Eupomatia
Austrobaileya
Chloranthac
Laurales
Winteraceae
Eudicots
Aristolochiac
Monocots
Piperales
Nymphaeales
Caytonia
Bennettitales
Pentoxylon
Glossopterids
Cycadales
Corystosperms
Autunia
Peltaspermum
Ginkgoales
Cordaitales
Emporia
Taxaceae
Cephalotaxus
Taxodiaceae
Araucariac
Podocarpac
Pinaceae
Ephedra
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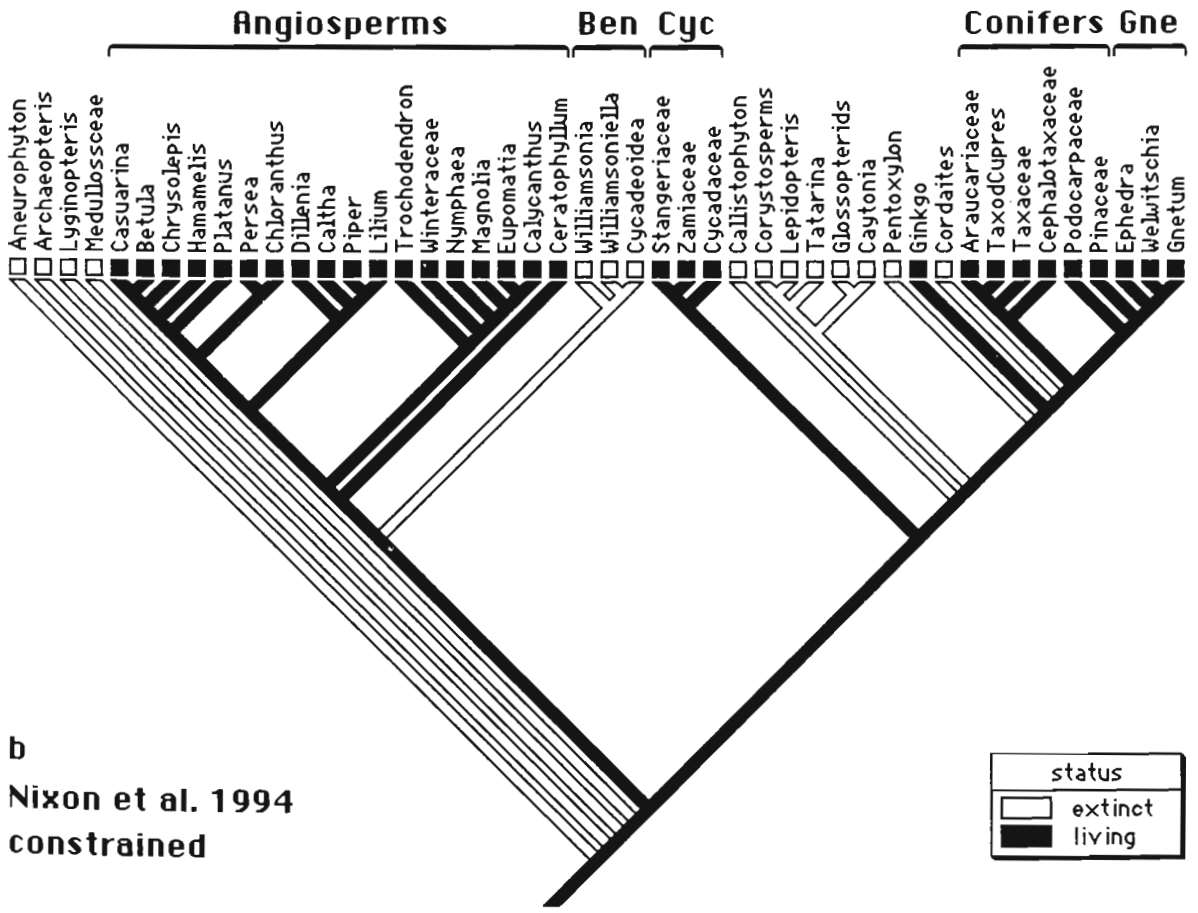
Angiosperms
Ben
Cyc
Conifers
Gne
Aneurophyton
Archaeopteris
Lyginopteris
Medullosseae
Casuarina
Betula
Chrysolepis
Hamamelis
Platanus
Persea
Chloranthus
Dillenia
Caltha
Piper
Lilium
Trochodendron
Winteraceae
Nymphaea
Magnolia
Eupomatia
Calycanthus
Ceratophyllum
Williamsonia
Williamsoniella
Cycadeoidea
Stangeriaceae
Zamiaceae
Cycadaceae
Callistophyton
Corystosperms
Lepidopteris
Tatarina
Glossopterids
Caytonia
Pentoxylon
Ginkgo
Cordaites
Araucariaceae
TaxodCupres
Taxaceae
Cephalotaxaceae
Podocarpaceae
Pinaceae
Ephedra
Welwitschia
Gnetum
b
Nixon et al. 1994
constrained
status
extinct
living

angiosperms separated from the line to their closest living relatives, and the age of the most recent common ancestor of all living angiosperms, or the crown group. As discussed above, trees of the types in Figs 2c-e imply that the stem lineages of living gymnosperms and angiosperms split in the Carboniferous, but this does not mean that the angiosperm crown group is this old; it could have originated much more recently, and one could argue that the large number of apomorphies separating angiosperms from other groups would take a long time to accumulate. However, the molecular dates being considered here relate specifically to the age of the crown group.

Of course, the age of the angiosperms has also been a topic of controversy in paleobotany. Until the 1960s, many paleobotanists assumed that angiosperms originated long before the Cretaceous, based in large part on identifications of Cretaceous fossils (mostly leaves) with diverse and advanced extant taxa (Axelrod, 1952, 1970). However, this view was challenged by palynological studies, which showed that Early Cretaceous angiosperm pollen was less diverse and more primitive than expected, and that the order of appearance of pollen types agreed with the sequence of evolution inferred from studies of modern plants – monosulcate, as in magnoliids and monocots; tricolpate, the basic type for what are now called eudicots; tricolporate; and finally triporate (Doyle, 1969, 1978; Muller, 1970, 1981). Closer examination of the leaf record and discoveries of fossil flowers and fruits showed a similar pattern of rapid but orderly morphological diversification (Crane *et al.*, 1995; Doyle & Donoghue, 1993; Doyle & Hickey, 1976; Friis & Crepet, 1987; Friis *et al.*, 1994b; Hickey & Doyle, 1977; Upchurch, 1984). Barremian-Aptian mesofossil floras show surprisingly high species diversity, but they do not contradict the picture of low initial advancement (Friis *et al.*, 2000). At present, the oldest definite angiosperm fossils are reticulate monosulcate pollen grains from the Valanginian or Hauterivian (ca. 135 Ma; Brenner, 1996; Hughes, 1994; Trevisan, 1988); a supposedly Jurassic record from China (*Archaefructus*: Sun *et al.*, 1998) has been redated as Barremian-Aptian (Barratt, 2000; Swisher *et al.*, 1999). These data suggest that angiosperms may have originated not long before their appearance in the fossil record, although they do not rule out the existence of older angiosperms if these were rare and plesiomorphic.

Molecular studies on this question have used the concept of a molecular clock, which assumes that gene sequences diverge at a statistically constant rate, to date splits between living groups. This requires at least one calibration point, a split either inside or outside the group that can be dated with the fossil record. Using the gene *gapC* and a rate of molecular evolution inferred from animals, Martin *et al.* (1989) dated the angiosperms, represented by two grasses and seven dicots, as 319 Ma, or mid-Carboniferous. At that time, the most advanced known seed plants were seed ferns more primitive than any living gymnosperms, to say nothing of angiosperms. Martin *et al.* took this result as support for the views of Axelrod (1952, 1970) and dismissed the concept of a Cretaceous origin as based on negative evidence. However, Crane *et al.* (1989a) argued that the conflict with the fossil record is not so easy to explain away. In particular, Martin *et al.* dated the common ancestor of eudicots as 276 Ma (Permian), but eudicots (which are strongly supported as a monophyletic group: Chase *et al.*, 1993; Qiu *et al.*, 1999; Soltis *et al.*, 1999) are united by tricolpate pollen, which has a dense fossil record, appearing in the late Barremian (Doyle, 1992; Hughes, 1994; Hughes & McDougall, 1990) and becoming ubiquitous in the Albian. Martin *et al.* (1989) did not use any calibration from the plant fossil record, but Martin *et al.* (1993) corrected this deficiency in a study of *gapC* and *rbcL*, which assumed that liverworts split from other land plants at 450 Ma (Late Ordovician); this gave an age of 300 Ma, again Carboniferous. However, younger (though still pre-Cretaceous) ages were found by Wolfe *et al.* (1989) and Laroche *et al.* (1995) - 200 Ma, or Early Jurassic - and by Goremykin *et al.* (1997), based on 58 chloroplast genes - 160 Ma, or Late Jurassic.

Analyses by Sanderson and Doyle (2001) suggest that these dates were biased upward by several factors, especially the fact that molecular evolution is not clocklike and the use of angiosperm taxa with higher than average rates. In addition, previous analyses assumed equal rates across DNA sites (which is known to be incorrect); correcting for this by use of a gamma distribution of rates gives angiosperm ages that are 20-30 Ma younger. Sanderson and Doyle (2001) used *rbcL* sequences from a larger number of taxa, chosen to span the base of the angiosperms and other important nodes. Fig. 4 shows one of their trees plotted against geologic time, calibrated with the divergence of *Marchantia* at 450 Ma, with branch lengths adjusted by a maximum-likelihood program to make molecular evolution as clocklike as possible, and using a gamma distribution. As in other analyses of *rbcL*, Gnetales were basal in seed plants. The estimated age of the angiosperms was 139 Ma, or earliest Cretaceous, close to their first appearance in the fossil record.

A problem with this analysis is that the seed plant relationships in Fig. 4 conflict with other data (as summarized above). Surprisingly, this has little effect on age estimates for angiosperms. When angiosperms and Gnetales were constrained to form a clade, as in the anthophyte hypothesis, the inferred age of angiosperms was 143 Ma, only 4 Ma from that in the previous tree, and the same age was found when Gnetales were forced together with conifers.

These results suggest that ages based on *rbcL* may be more compatible with the fossil record than has been thought. However, ages using the same methods based on 18S rDNA (Sanderson & Doyle, 2001) were still substantially older, varying around 180-190 Ma (depending on seed plant relationships), or Early Jurassic.

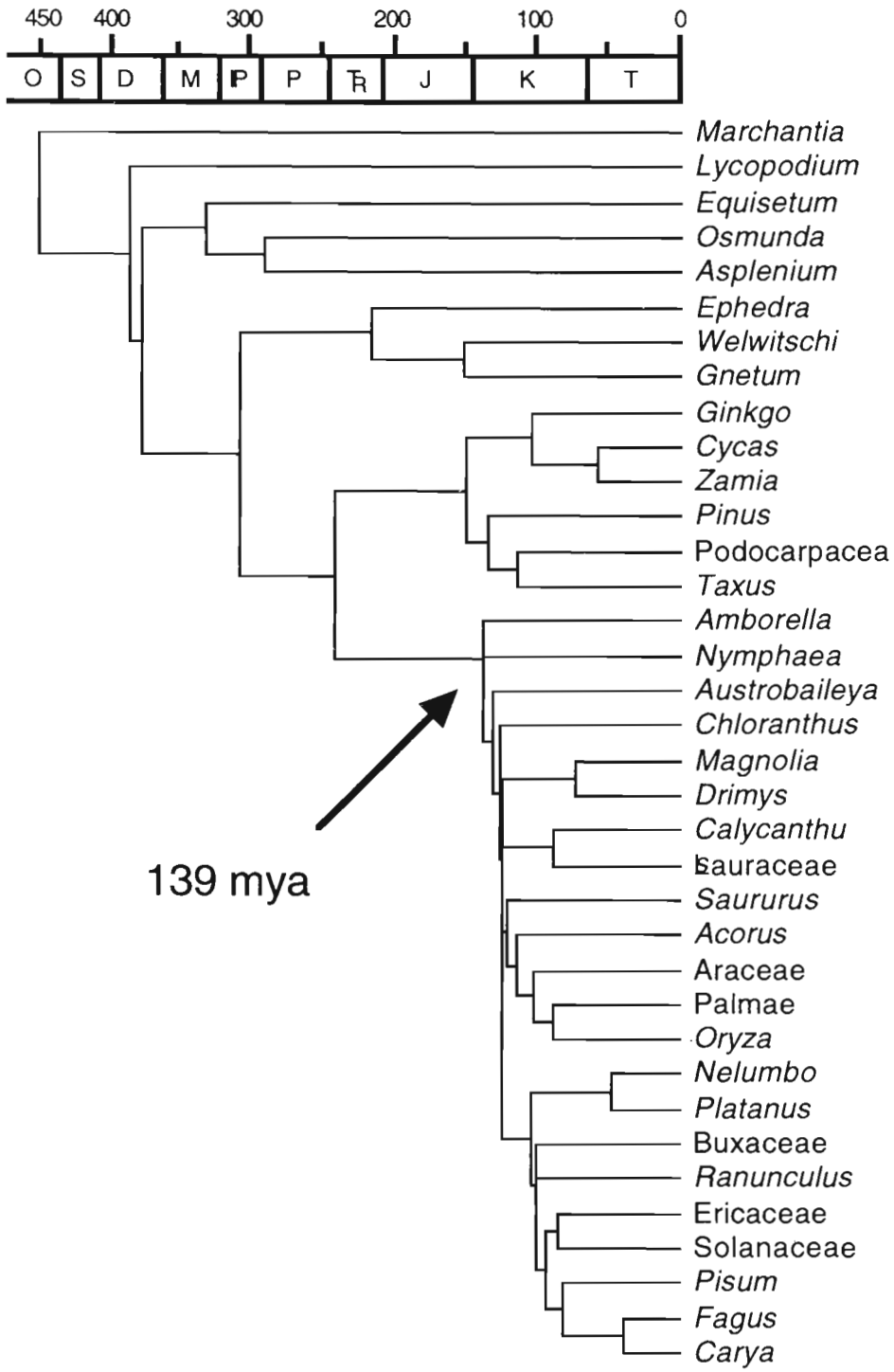

Fig. 4—Seed plant tree based on *rbcL*, with *Lycopodium* forced to the base of vascular plants and conifers forced into a clade, plotted against the geologic time scale, with ages estimated from *rbcL* by maximum likelihood under the assumption of a molecular clock (Sanderson & Doyle, 2001).

Closer examination of the *rbcL* data suggests reasons why previous analyses gave older ages, while warning that the problems are far from solved. Although the ages of angiosperms obtained by Sanderson and Doyle (2001) are consistent with the fossil record, evolution was clearly not clocklike across the tree. In some cases, this gave ages that are too young. Fig. 4 indicates that cycads, *Ginkgo* and conifers split in the Late Jurassic (152 Ma), whereas in fact

they go back twice as far, to the Late Carboniferous or Permian. The reasons for this anomaly are clearer in Fig. 5, the same tree presented as a phylogram, with branch lengths proportional to the amount of molecular evolution. Cycads, *Ginkgo* and conifers are unusually short branches; apparently, in pulling the tips of these branches up to the same level as other groups, the likelihood program pulled up their common ancestor too. Some ages within angiosperms are also too young: the split between *Nelumbo* and *Platanus* was dated as 48 Ma, but the lines leading to both groups are known back to the Albian, 100-110 Ma. In contrast, other branches within angiosperms are unusually long—*Oryza*, *Pisum* and *Nicotiana*, all advanced herbaceous taxa. The fact that rates are higher in grasses was already noted by Bousquet *et al.* (1992) and Gaut *et al.* (1992). It happens that earlier clock studies were based largely on such cultivated plants. When Sanderson and Doyle (2001) used *Oryza*, *Pisum* and *Nicotiana* as the only angiosperms in their *rbcL* data set, the estimated age of the group almost doubled - to 253 Ma, or Late Permian.

Sanderson and Doyle (2001) found that age estimates also vary depending on codon positions, with third positions of *rbcL* actually giving angiosperm ages that are too young (Late Cretaceous), and first and second positions giving older ages than the whole sequence. Several previous studies (including Martin *et al.*, 1989, 1993) analyzed amino acid sequences or nonsynonymous substitutions, which involve mostly changes at first and second positions. When Sanderson and Doyle (2001) used *Oryza*, *Pisum* and *Nicotiana* as the only angiosperms on a tree with Gnetales linked with conifers and analyzed only first and second positions of *rbcL*, they obtained a date of 281 Ma, approaching the 300-320 Ma ages of Martin *et al.* (1989, 1993). Thus, taxon sampling and codon positions effects go far toward explaining the older ages obtained in previous studies. However, this does not indicate which estimates are more nearly correct.

The conflicting ages derived from different genes and the clear inequalities in evolutionary rate among lineages suggest that better understanding of factors influencing rates of molecular evolution and/or development of new methods that deal with unequal rates might reconcile fossil and molecular ages. Unfortunately, the most popular method proposed so far, nonparametric rate smoothing (NPRS; Sanderson, 1997), yields *rbcL* ages for angiosperms that are considerably older than ages based on a clock (Doyle *et al.*, 2001), actually aggravating the conflict. This could mean either that the fossil record is more incomplete than clock-based estimates imply, or that rates of molecular evolution change abruptly rather than gradually (as assumed by NPRS), so that NPRS is even less appropriate than the clock method. These problems should be a topic of continued dialogue between paleobotanists and molecular evolutionists.

MOLECULAR PHYLOGENIES AND CRETACEOUS ANGIOSPERMS

While molecular data suggest we know less about the outgroups and the age of the angiosperms than we thought, they have greatly clarified the third question - what the first angiosperms were like. In cladistic terms, this is a function of rooting of the angiosperm tree, which depends on character states in the outgroups. In the period of morphological cladistics, it seemed that this problem might not be solved without clear identification of angiosperm outgroups. For example, Donoghue and Doyle (1989) rooted the angiosperms with a hypothetical ancestor based on the seed plant analysis of Doyle and Donoghue (1986). This indicated that Magnoliales were basal in angiosperms, as a result of granular exine structure and other states that they share with Bennettitales and Gnetales, the supposed closest outgroups of angiosperms. In contrast, in trees of Nixon *et al.* (1994; Fig. 1a), in which angiosperms were nested in Gnetales, Chloranthaceae were basal, consistent with their opposite leaves, simple flowers and orthotropous ovules, all gnetalian features. In trees of Doyle (1996; Fig. 1b), in which *Caytonia* was the sister group of angiosperms, Nymphaeales were basal.

In contrast, molecular analyses have converged remarkably on similar rootings of the angiosperms, despite the uncertainties on outgroup relationships discussed above. The main exception was the first large analysis, using *rbcL* (Chase *et al.*, 1993), which placed the aquatic genus *Ceratophyllum* at the base of the angiosperms. The first signs of the present picture came from studies of nuclear rRNA (Doyle *et al.*, 1994; Hamby & Zimmer, 1992) and chloroplast rDNA ITS sequences (Goremykin *et al.*, 1996), which indicated that Nymphaeales were basal. More recent multigene studies have kept Nymphaeales near the base while adding several other taxa around them. The data used included various combinations of *rbcL* and *atpB* from the chloroplast, 18S rDNA from the nucleus, and five mitochondrial genes (Barkman *et al.*, 2000; Parkinson *et al.*, 1999; Qiu *et al.*, 1999; Soltis *et al.*, 1998, 1999, 2000); duplicated phytochrome genes (Mathews & Donoghue, 1999); and 17 chloroplast genes (Graham & Olmstead, 2000). In most of these studies, the first branch was *Amborella*, a vesselless shrub from New Caledonia formerly placed in Laurales; the second was Nymphaeales; and the third was a clade consisting of Illiciales and two Australasian lianas, *Austrobaileya* and *Trimenia* (placed in their own families). These lines were called the 'ANITA grade' by Qiu *et al.* (1999). The main uncertainty concerns the exact relationship of *Amborella* and Nymphaeales. Their placement as successive branches has low bootstrap support and is sensitive to taxon sampling (Graham & Olmstead, 2000; Qiu *et al.*, 2000), and analyses using the RASA method, designed to counteract long-branch attraction, unite *Amborella* and Nymphaeales as a basal clade (Barkman

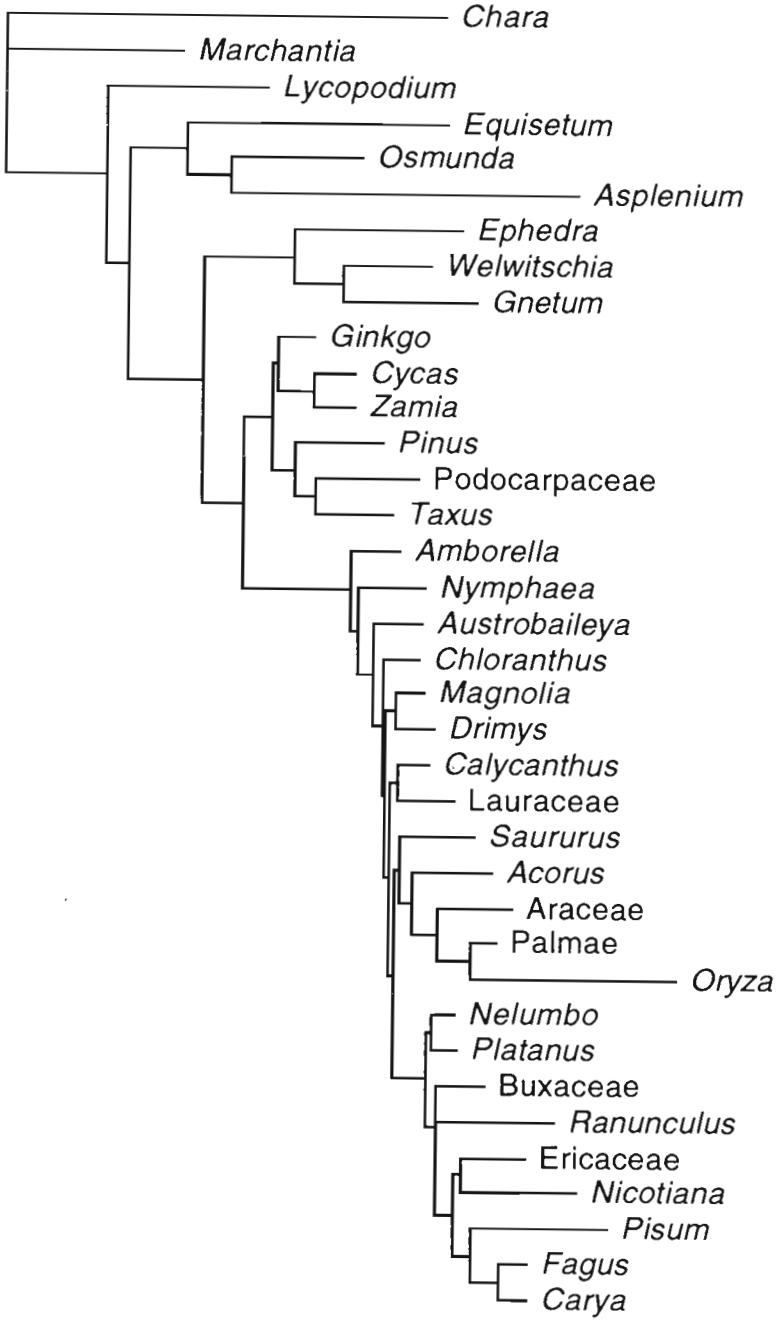

Fig. 5—Tree in Fig. 4 presented as a phylogram, with branch lengths proportional to the amount of molecular evolution (Sanderson & Doyle, 2001).

et al., 2000; contrary to these authors, this has only minor effects on inferred ancestral states). Above the ANITA grade, there are eight major clades, all of which have high bootstrap support, but whose mutual relationships are not completely resolved. Thus, even though living gymnosperms are very distant from angiosperms and their arrangement is uncertain, they appear to provide a strong molecular signal for rooting the angiosperms.

These molecular trees show striking parallels with the Cretaceous fossil record. This point can be illustrated by plotting characters on the tree in Fig. 6, from a study by Doyle and Endress (2000), who combined a new morphological data set with *rbcL*, *atpB* and 18S sequences and specified *Amborella* as the outgroup to other taxa. This 'combined' tree resembles molecular trees in most respects, except in a few places where molecular support was weak. For example, it

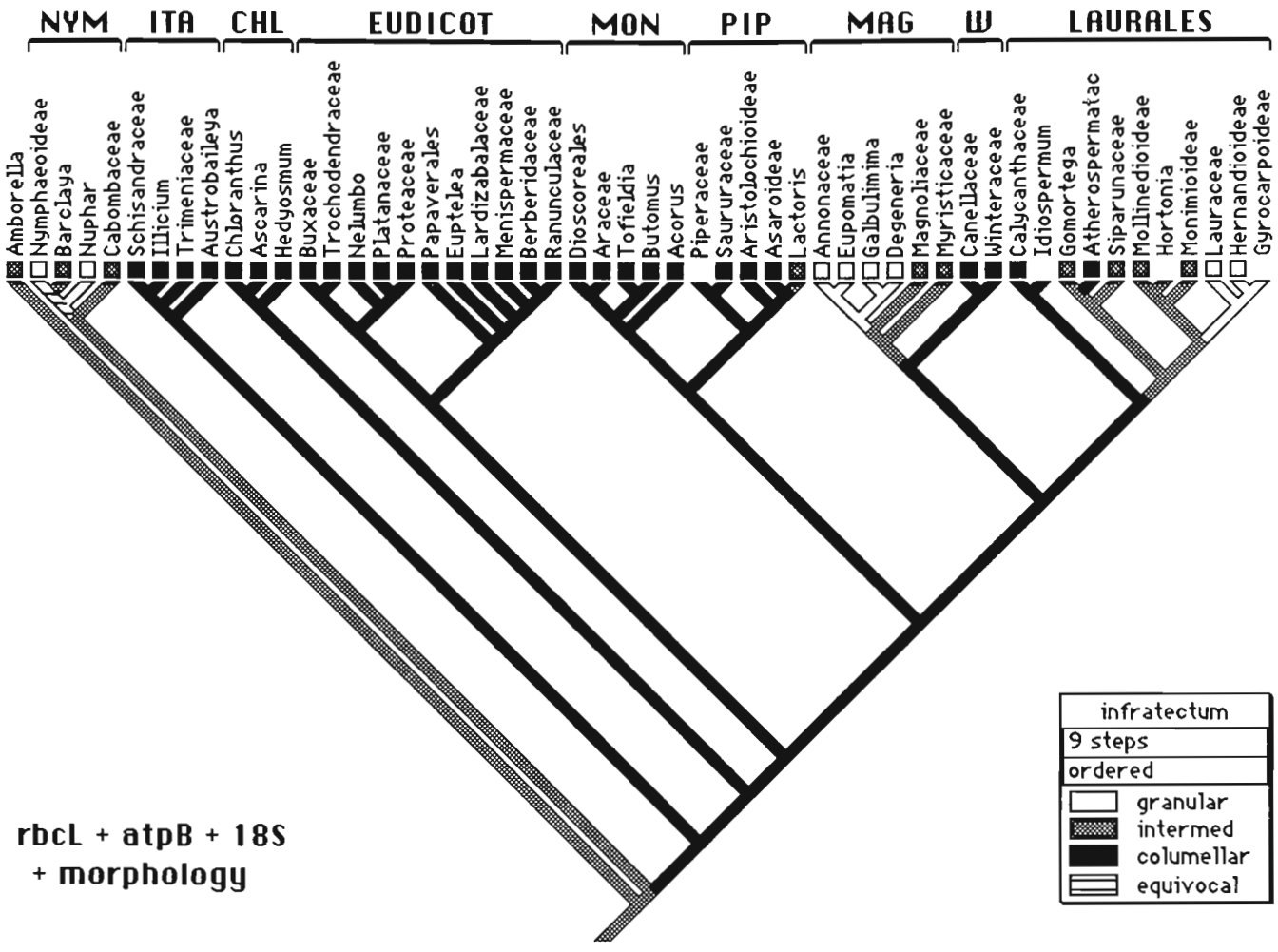

Fig. 6— Single tree found in the combined analysis of Doyle and Endress (2000), based on morphology, *rbcL*, *atpB* and 18S rDNA, showing the inferred evolution of exine structure. CHL = Chloranthaceae, MAG = Magnoliales, W = Winterales, PIP = Piperales, NYM = Nymphaeales, MON = monocots.

links monocots with Piperales (*sensu* APG, 1998, including Aristolochiaceae and *Lactoris*), Winterales with Magnoliales rather than Piperales and Lauraceae with Hernandiaceae rather than Monimiaceae (Monimioideae, *Hortonia*, Mollinedioideae).

Perhaps most interesting is the case of Chloranthaceae, which have occupied several positions in molecular trees, but which morphology helps to place immediately above the ANITA grade. This near-basal position is consistent with the abundance of apparent fossil relatives of Chloranthaceae in the Early Cretaceous, such as the *Clavatipollenites* and *Asteropollis* pollen groups (Couper, 1958; Muller, 1981; Walker & Walker, 1984), flowers and fruits associated with these pollen types (Eklund *et al.*, 1997; Friis *et al.*, 1986, 1994b, 1999; Pedersen *et al.*, 1991), and probably some of the leaves with chloranthoid teeth discussed below. Some Barremian-Aptian fossils (Friis *et al.*, 1994b, figs 3c-f; Friis *et al.*, 1997a, fig. 6.3) appear to be related to the living genus *Hedyosmum*: both groups have pollen with a branched sulcus (*Asteropollis* in the dispersed pollen record) and three tepals fused to the carpel, indicating that the crown group of Chloranthaceae had evolved by this time. Tripartite androecia related to *Chloranthus* are diverse in the Late Cretaceous, and a more problematic example (because of the anomalous orientation of the anthers) is known from the Albian (Crane *et al.*, 1989b; Eklund *et al.*, 1997; Friis *et al.*, 1986; Herendeen *et al.*, 1993).

The new molecular rooting is also significant in suggesting that the earliest Cretaceous angiosperms may be closer to the origin of the clade than previous phylogenetic views implied. A prime example concerns exine structure. The oldest generally accepted angiosperm pollen grains, from the Valanginian of Italy (Trevisan, 1988), the Valanginian-Hauterivian of Israel (Brenner, 1996) and the Hauterivian of

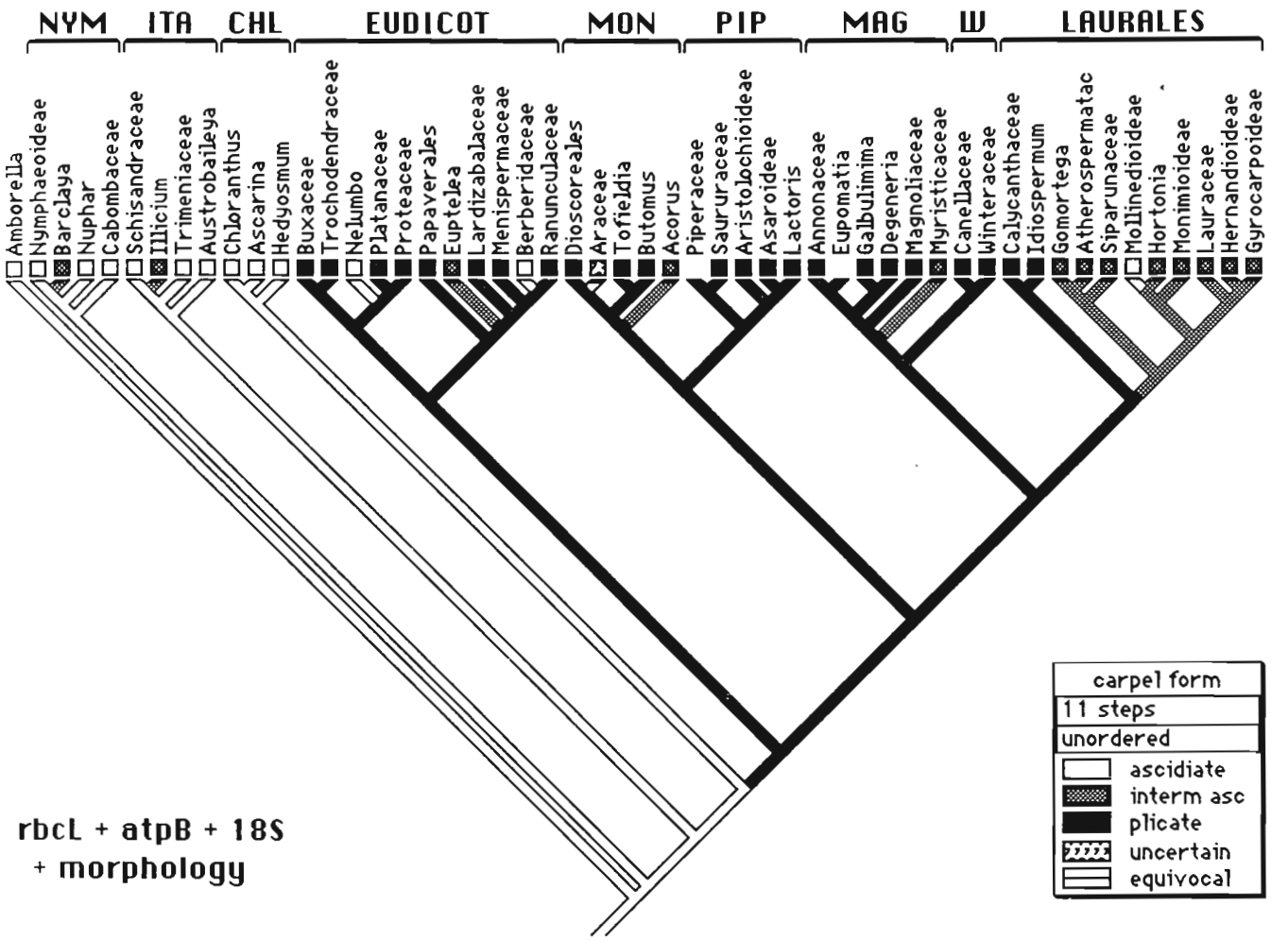

Fig. 7—Tree from the combined analysis of Doyle Endress (2000), showing the inferred evolution of carpel form

England (Hughes, 1994; Hughes & McDougall, 1987; Hughes *et al.*, 1991), are monosulcates and inaperturates with reticulate sculpture and columellar exine structure. It has been argued that the plants producing this pollen were already advanced relative to Magnoliales with smooth pollen and granular exine structure (Muller, 1970; Van Campo & Lugardon, 1973; Walker, 1976; Walker & Walker, 1984; Ward *et al.*, 1989), and this view seemed to be supported by the basal position of Magnoliales in the cladistic analysis of Donoghue and Doyle (1989). Such pollen would be hard to distinguish from that of Bennettitales without TEM study, and it could extend back much earlier without being recognized. However, molecular trees (Fig. 6) indicate that granular structure is actually derived in angiosperms, since Magnoliales and other granular taxa are nested within the group, not basal. The inferred ancestral structure, retained in some Nymphaeales (Cabombaceae, *Barclaya*), had irregular columellae overlain by a continuous tectum; this was called intermediate by Doyle and Endress (2000) but columellar by Osborn *et al.* (1991). Typical columellar structure originated at the third node, along with a reticulate tectum, resulting in pollen like that of *Austrobaileya* (Endress & Honegger, 1980), which would be at home in the Hauterivian. Hence there is no longer reason to assume a long period of angiosperm evolution before the appearance of such pollen.

This conclusion holds all the more if *Amborella* is linked with Nymphaeales (Barkman *et al.*, 2000), since under this arrangement it is equally parsimonious to assume that either tectate-intermediate or reticulate-columellar exines were ancestral. The discovery by Friis *et al.* (2001) of a Barremian-Aptian flower similar to Nymphaeales but with reticulate-columellar pollen may be evidence for the latter view. However, even if the first angiosperm pollen was tectate, the molecular results suggest that it might be more recognizable than pollen of Magnoliales. *Amborella* has monosulcate pollen with verrucate sculpture, small supratectal spinules and sparse, irregular columellae (Hesse, 2001; Sampson, 1993). Hughes

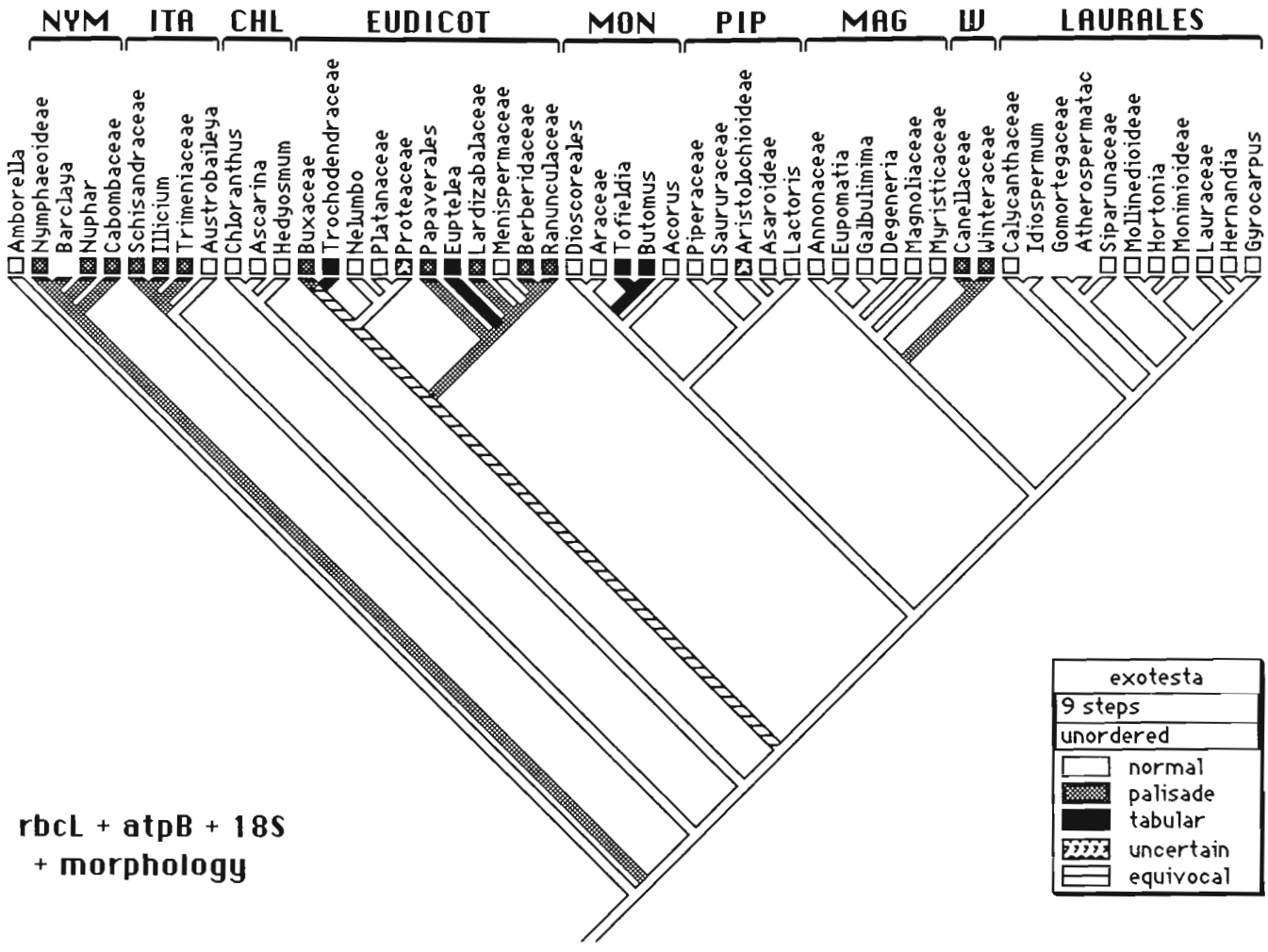

Fig. 8—Tree from the combined analysis of Doyle and Endress (2000), showing the inferred evolution of exotestal structure.

and McDougall (1987) and Hughes (1994) described pollen with almost identical sculpture from the Hauterivian of England as HAUTERIVIAN-CACTISULC. It would be unwarranted to identify this pollen as *Amborella*, but it does show that such pollen existed and can be detected in the earliest angiosperm record.

Other agreements between molecular trees and the Cretaceous record concern carpel morphology. The classical view (especially among American botanists) is that the original carpel was plicate (conduplicate), like a leaf folded down the middle, as in *Degeneria* and Winteraceae (Bailey & Swamy, 1951). However, molecular trees imply that the ancestral carpel was ascidiate, as proposed by Leinfellner (1969) and van Heel (1981). Both carpel types begin their development as a U-shaped primordium. In the plicate type, the two arms of the primordium grow up separately, but in the ascidiate type the cross-zone between the arms becomes meristematic, and the carpel grows up like a tube. At maturity ascidiate carpels are typically barrel-shaped, with a sessile stigma, and they are sealed by secretion rather than postgenital fusion of the margins (Endress & Igersheim, 2000). The combined tree of Doyle and Endress (2000) indicates that the ascidiate state was ancestral and retained up to Chloranthaceae (Fig. 7). This agrees with the fact that most carpels reported by Friis *et al.* (1994b, 1999, 2000) from the Barremian-Aptian of Portugal appear to be ascidiate, judging from their shape, sessile stigma, and lack of evidence for a ventral suture, including both those associated with Chloranthaceae and others. At the fruit stage most had one seed, like *Amborella*, *Trimenia*, *Illicium*, and Chloranthaceae, but a few had several, like other ANITA taxa (Nymphaeales, *Austrobaileya*, Schisandraceae). Friis *et al.* (2000) suggested that both ascidiate and plicate carpels were present, with the latter represented by follicular fruits, but follicles were less common than berries, drupes, nuts and achenes (Eriksson *et al.*, 2000).

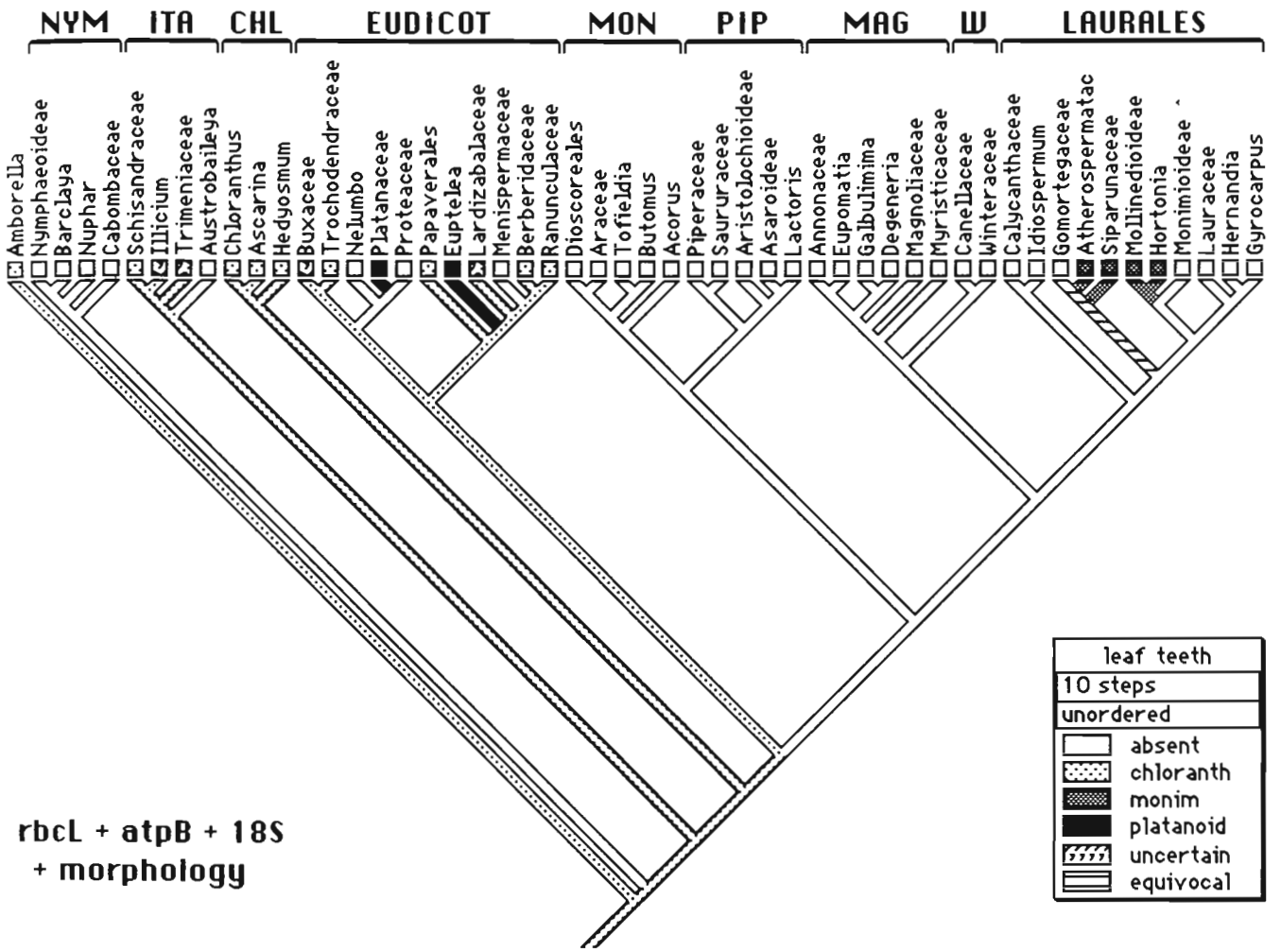

Fig. 9—Tree from the combined analysis of Doyle and Endress (2000), showing the inferred evolution of leaf margin.

Another conspicuous feature of Barremian-Aptian mesofloras is exotestal seed structure, in which cells of the outer epidermis of the outer integument become thick-walled (Friis *et al.*, 1999, 2000). This feature is typical of Nymphaeales, *Trimenia* and Illiciales. It is not inferred to be ancestral on the cladogram in Fig. 8, since it is absent in *Amborella* and *Austrobaileya*, but this may be a function of the relict and specialized (autapomorphic) nature of living ANITA taxa. *Amborella* is autapomorphic in having a hard endocarp derived from the inner carpel wall, *Austrobaileya* in having a fleshy sarcotesta derived from the mesophyll of the outer integument. For functional reasons, origin of either feature might be expected to entail loss of a hard, protective exotesta if this was present in the first angiosperms.

As stressed by Friis *et al.* (2000), Barremian-Aptian mesofloras consist largely of taxa that cannot be associated with any one extant family (except for Chloranthaceae). Many may therefore represent extinct lines on the stem lineages of modern families or the internodes between them. However, as the comparisons made here show, this does not prevent them from contributing to formulation and testing of hypotheses on basic states and character evolution in angiosperms, through comparison with molecular phylogenies and improved data on the distribution of morphological characters.

Similar leaf features are also found at the base of molecular trees and in early angiosperms. An example mentioned above is chloranthoid teeth (Hickey & Wolfe, 1975), with three veins joining below a cap-like apical gland. These occur in modern Chloranthaceae and Barremian-Aptian leaves, such as DBLT no.1 of Upchurch (1984) from the lower Potomac Group (Aptian?) and *Moutonia* spp. of Pons (1984) from the late Barremian or Aptian of Colombia. However, as shown in Fig. 9, chloranthoid teeth are found not only in Chloranthaceae, but also in several ANITA groups and basal eudicots (cf. Hickey & Wolfe, 1975), and Fig. 9 implies that they are ancestral for angiosperms.

Lower Potomac leaves are also notable for their variable stomata, which often vary from paracytic to laterocytic to cyclocytic on the same leaf (Upchurch, 1984). Upchurch suggested that this variation was primitive, like the irregular ('first rank') venation of lower Potomac leaves (Doyle & Hickey, 1976; Hickey & Doyle, 1977; Wolfe *et al.*, 1975). In surveying extant magnoliids, Upchurch (1984) found similar variation in only a few taxa, notably *Amborella*, *Austrobaileya*, *Schisandra* and Chloranthaceae. At the time, this list did not seem particularly significant, but these taxa now stand out as groups located near the base of molecular and combined trees.

The next phases of the angiosperm record also agree with molecular phylogenies by showing new types that can be related to several clades above the ANITA grade, often to basal members of these clades. Among the oldest are monoporate tetrads from the late Barremian of Gabon and the Aptian-Albian of Israel, which resemble Winteraceae in the tetrad habit and ring of endexine around the pore but appear to be more primitive in having finer sculpture (Doyle, 2000; Doyle *et al.*, 1990a, b; Walker *et al.*, 1983).

Tricolpate pollen, which is diagnostic for the eudicot clade, is first seen in the probable late Barremian of Gabon and England (Doyle, 1992; Doyle *et al.*, 1977; Doyle & Hotton, 1991; Hughes, 1994; Hughes & McDougall, 1990), and it has been found in stamens from the Barremian-Aptian of Portugal (Friis *et al.*, 1994b, 2000). The first tricolpates from Gabon have irregularly arranged furrows (Doyle, 1992; Doyle & Hotton, 1991), suggesting they may be on the eudicot stem lineage rather than in the crown group (although similar irregularities occur in the near-basal eudicot *Nelumbo*: Borsch & Wilde, 2000; Kuprianova, 1979). In the lower Potomac Group (Aptian?), there are ternately lobed leaves called *Vitiphyllum* (Berry, 1911; Doyle & Hickey, 1976; Hickey & Doyle, 1977) that are suggestive of Ranunculales, the first eudicot branch in molecular trees.

Other lower Potomac fossils have been compared with monocots (Doyle, 1973; Walker & Walker, 1984): narrow leaves with apically converging venation, known as *Acaciaephyllum*; and monosulcate pollen called *Liliacidites*, with sculpture that grades from coarse in the middle of the grain to fine at the ends, a feature found in many monocots but not known in living magnoliids. These comparisons have been criticized by Gandolfo *et al.* (2000). These authors were correct in arguing that the leaf criteria proposed by Doyle (1973) for separating monocots from gymnosperms do not separate monocots from Gnetales, including the lower Potomac genus *Drewria* (Upchurch & Crane, 1985). Still, *Acaciaephyllum* appears to differ from *Drewria* and extant Gnetales in having spiral rather than opposite leaf arrangement and apical vein fusion. Some pollen types assigned to *Liliacidites* and considered monocots by Doyle (1973) and Walker & Walker (1984), but segregated by Góczán and Juhász (1984) as *Similipollis*, differ in having finer sculpture at the proximal pole and sulcus margins rather than the ends of the grain and were therefore questioned as monocots by Doyle and Hotton (1991). As noted by Gandolfo *et al.* (2000), Friis *et al.* (1997b) associated such pollen with carpels (*Anacostia*) that were clearly not monocots (possibly related to Illiciales?). However, more distinctively monocot-like pollen with fine sculpture at the ends of the grain has not yet been found *in situ*. Gandolfo *et al.* cited several other associations of *Liliacidites* with non-monocotyledonous floral remains (e.g., *L. minutus*, associated with *Virginianthus*, discussed below), but all of these are pollen types without sculpture gradation that were assigned to *Liliacidites* by authors who used this genus in a broader sense.

Floral remains representing another magnoliid line, Laurales, are known from the Albian. *Virginianthus* (Friis *et al.*, 1994a), from the upper Potomac Group, appears to be related to Calycanthaceae in having a deep hypanthium and other calycanthaceous features but is more primitive in having monosulcate rather than disulculate pollen. Cenomanian flowers and inflorescences called *Mauldinia* (Drinnan *et al.*, 1990) and associated wood (*Paraphyllanthoxylon*; Herendeen, 1991) correspond in great detail to Lauraceae, and similar but more fragmentary flowers occur in the Albian (Crane *et al.*, 1994). Magnoliales, once considered the most primitive angiosperms but not basal in molecular trees, are not definitely known until the Cenomanian, represented by flowers and leaves of *Archaeanthus* (Dilcher & Crane, 1984), an apparent stem relative of Magnoliaceae. However, Aptian monosulcate pollen with granular exine structure (*Lethomasites*; Ward *et al.*, 1989) and Albian laminar stamens containing smooth monosulcate pollen (Crane *et al.*, 1994; figs 11a, b) may represent this clade.

The Albian marks the explosion of tricolpate eudicot pollen, and many Albian megafossils can be associated with particular eudicot lines. Significantly, all well-reconstructed Albian eudicots appear to be related to groups that molecular phylogenies place near the base of the clade. Many are relatives of *Platanus* and *Nelumbo*, which molecular data unexpectedly unite with Proteaceae in a clade called Proteales (APG, 1998), probably the second branch in eudicots. The oldest are inflorescences of unisexual flowers that resemble those of *Platanus* but are associated with pinnately compound *Sapindopsis* leaves (Crane *et al.*, 1993), and peltate *Nelumbites* leaves and pitted floral receptacles that differ from those of *Nelumbo* in being round rather than flat-topped (Upchurch *et al.*, 1994). Relatives of Buxaceae, another early branch, are represented by inflorescences of unisexual flowers called *Spanomera* (Drinnan *et al.*, 1991). It is not until the Cenomanian that we see definite 'core' eudicots (rosids, asterids, and associated smaller groups), such as pentamerous flowers of a rosid type (Basinger & Dilcher, 1984) and the first members of the Normapolles pollen complex (Laing, 1975; Pacltová, 1971, 1981), later members of which have been associated with flowers of 'core Amentiferae' or 'higher' Fagales (Friis, 1983; Schönenberger *et al.*, 2001; Sims *et al.*,

1999). However, tricolporate pollen, which is probably basic for rosids and asterids, is known from the late Albian (Doyle & Robbins, 1977; Laing, 1975), suggesting that core eudicots had originated by that time.

The fact that the stratigraphic pattern of appearance of pollen, leaf and floral types agrees as well as it does with molecular phylogenies may also be an argument against molecular clock analyses that date the angiosperms as much older than the Cretaceous. If angiosperms had undergone a substantial part of their diversification before the Cretaceous, it is hard to imagine why they 'waited in line' and then filed into the Cretaceous fossil record in the same order that molecular evidence indicates they originated long before.

ANGIOSPERM ROOTING AND ANGIOSPERM OUTGROUPS

Although molecular trees do not indicate directly which fossil seed plants were most closely related to angiosperms, they may help indirectly in the search for angiosperm outgroups by suggesting which states are ancestral and thereby refining our search image. For example, the discovery of vessels in Permian gigantopterids (Li & Taylor, 1999; Li *et al.*, 1996) might suggest that these plants were related to angiosperms. However, molecular results (cf. Fig. 6) contradict this argument, since (unlike earlier morphological analyses: Donoghue & Doyle, 1989; Young, 1981) they indicate that the common ancestor of angiosperms did not have vessels: *Amborella* is vesselless, and Nymphaeales either lack vessels or have cells with porose pit membranes that are intermediate between tracheids and vessel elements (Schneider & Carlquist, 1996; Schneider *et al.*, 1995). In contrast, however, the same trees imply that the absence of vessels in Winteraceae and Trochodendraceae is a result of secondary loss. Ecological and biogeographic scenarios for such loss, as a possible consequence of migration into cooler high-latitude areas, have been discussed by Doyle (2000) and Feild *et al.* (2000).

Another example concerns exine structure. As shown in Fig. 6 and discussed above, molecular phylogenies imply that the first angiosperms had columellar structure (either intermediate or well-developed), rather than granular structure, as previously thought (Donoghue & Doyle, 1989; Van Campo & Lugardon, 1973; Walker, 1976; Walker & Walker, 1984). This suggests that paleobotanists should look more closely at Late Triassic monosulcate and related pollen types described by Cornet (1989) as the Crinopolles group, which have reticulate sculpture and conspicuous columellae but a thick, gymnosperm-like endexine (Cornet, 1989; Doyle & Hotton, 1991). Doyle and Hotton (1991) argued that this combination of features might mean that Crinopolles plants were related to but more primitive than angiosperms (i.e., angiosperm stem relatives). This suggestion conflicted with the hypothesis that the first angiosperms had granular exines, which predicted that the gymnospermous endexine was lost before the origin of columellae, but the molecular evidence that columellar exines are ancestral removes this objection. Thus association of Crinopolles pollen with other organs should be a high priority for paleobotanists.

Although it would be most sensational to find angiosperm stem relatives in the Triassic or Jurassic, the possibility that some such plants persisted into the Cretaceous should not be overlooked. A possible candidate is *Archaefructus*, originally described as Late Jurassic (Sun *et al.*, 1998) but redated as Barremian-Aptian (Barratt, 2000; Swisher *et al.*, 1999), an elongate axis bearing numerous well-spaced follicles. If *Archaefructus* is a floral axis, as assumed by Sun *et al.* (1998), it is unlike and possibly more primitive than anything in living angiosperms. However, if it is instead an inflorescence, this argument may not hold. Another is *Afropollis* (Doyle *et al.*, 1982), a widespread pollen group in the late Barremian through Cenomanian of Northern Gondwana, which includes coarsely reticulate grains that vary from operculate to zonasulculate and inaperturate. Doyle *et al.* (1990a, b) speculated that *Afropollis* might represent extinct relatives of Winteraceae, but unlike most angiosperms and like the Crinopolles it has a thick, laminated endexine. Friis *et al.* (1999) found *Afropollis* in non-angiospermous microsporangia from the Barremian-Aptian of Portugal, apparently excluding it from the angiosperm crown group, but not necessarily from the stem lineage (Doyle, 2000).

CONCLUSION

It would be presumptuous to argue that paleobotanists should accept molecular phylogenies without question, but these trees are based on a vast and ever-growing body of data that cannot be ignored, and they can be a rich source of hypotheses for future research. Furthermore, molecular trees show remarkable agreements with the Cretaceous angiosperm record. Depending on which line of evidence is considered more reliable, this congruence can be taken as fossil confirmation of molecular results, molecular evidence that the fossil record provides a good picture of the early phases of angiosperm evolution, or both.

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