THE PLANT TREE OF LIFE: AN OVERVIEW AND SOME POINTS OF VIEW[1]

JEFFREY D. PALMER,[2,5] DOUGLAS E. SOLTIS,[3] AND MARK W. CHASE[4]

[2]Department of Biology, Indiana University, Bloomington, Indiana 47405-3700 USA; [3]Department of Botany and the Genetics Institute, University of Florida, Gainesville, Florida 32611 USA; and [4]Molecular Systematics Section, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, TW9 3DS, United Kingdom

We provide a brief overview of this special issue on the plant tree of life, describing its history and the general nature of its articles. We then present our estimate for the overall topology and, for land plants, divergence times of the plant tree of life. We discuss several major controversies and unsolved problems in resolving portions of this tree. We conclude with a few thoughts about the prospects for obtaining a comprehensive, robustly resolved, and accurately dated plant tree of life and the importance of such a grand endeavor.

Key words: algae; endosymbiosis; Gnepines hypothesis; land plants; phylogeny; plastids.

To mark the 90th anniversary of this journal, Scott Russell, the then-Immediate Past President of the Botanical Society of America, and Karl Niklas, the long-term Editor-in-Chief of the *American Journal of Botany*, jointly asked the three of us to serve as guest editors for a special issue devoted to the remarkable recent progress in reconstructing the evolutionary history of plant life, also known as the plant tree of life. Our invitations to prospective authors went out in June of 2003. For this issue to appear in print only 16 months later, with 100% delivery of solicited articles and each of them outstanding, is nothing short of remarkable. This testifies to the commitment and enthusiasm of the authors; to the editorial expertise and dedication of Karl Niklas, the journal's office manager Caroline Spellman, copy editor Beth Hazen, and production editors Beth Hazen and Elizabeth Lawson; and to the timely cooperation of the two to four expert scientists chosen to provide rigorous, anonymous, and speedy review of each article. Although the rapid gestation of this issue ensures that it is up-to-date and timely, these articles should also, we believe, prove to be of relatively long-lasting and enduring value. We trust that readers of this first-ever special issue of this journal will be as delighted with the outcome of this effort as are we.

NATURE OF THE ARTICLES IN THIS ISSUE

The other 18 articles in this issue fall into two general categories. Twelve are taxon-oriented, while the other six are topical in nature. Of the first set, 11 are taxon-specific, providing broad coverage of current understanding of the phylogeny and overall evolution of the major groups of "plants," as defined either phylogenetically or historically. The twelfth, by Keeling (2004), was commissioned to provide a broad overview of eukaryotic phylogeny and plastid primary and secondary symbiosis. Such an overview is essential to have proper perspective on the origin and phylogenetic relationships of land plants and the many diverse groups of "algae," as well as those otherwise unrelated groups (especially fungi) that nonetheless have traditionally been allied with plants and which are still part of the purview of this journal and its governing society. The group-specific authors were given free rein as to how to approach and cover their groups. Thus, you will see a wide range of treatments. The central, common thread, however, to all 11 of these articles is a description, usually encompassing a critical assessment, of our current state of understanding of the phylogeny of each group. Although this phylogenetic understanding increasingly relies on gene sequence data, many of the articles also provide a more or less thorough integration of phylogeny with the overall evolution (principally from a morphological standpoint) and classification of the group in question.

The coverage represented by the 11 taxon-specific papers reflects the amount of effort and progress made in elucidating the phylogeny of each group, as well as their size and economic and ecological importance. Thus, angiosperms, the largest, most important, and best studied group of land plants, are covered by three articles (Soltis and Soltis, 2004; Chase, 2004; Judd and Olmstead, 2004), whereas three much smaller but considerably older groups of land plants—seed plants (Burleigh and Mathews, 2004), ferns (Pryer et al., 2004), and bryophytes (Shaw and Renzaglia, 2004)—are each given but a single article. Likewise, green algae, the parent group of land plants, are treated in a single paper (Lewis and McCourt, 2004), as are the sister group to green algae/land plants, the red algae (Saunders and Hommersand 2004). Two papers cover three of the largest and best studied groups of secondary plastid-containing algae, the dinoflagellates (Hackett et al., 2004) and the putatively related heterokonts (brown algae, diatoms, chrysophytes, etc.) and haptophytes (Andersen, 2004). Finally, Lutzoni et al. (2004) take on all the fungi. Page limits to this issue made for hard choices, and we regret that four fascinating but mostly poorly studied groups with secondary plastids (apicomplexans, chlorarachniophytes, cryptomonads, and euglenids), as well the primary plastid-containing glaucophytes, could be given only brief mention in the overview by Keeling (2004).

With three exceptions, all 12 taxon-oriented articles are es-

sentially review articles, providing a critical overview and synthesis of the recent literature pertinent to their respective subjects. The article by Lutzoni et al. (2004) on fungal phylogeny is the first paper from a large team of investigators whose currently funded goal (from the U.S. National Science Foundation Tree-of-Life Program) is to reconstruct phylogenetic relationships of 1500 fungi using eight genes (ca. 10 kb of DNA sequence) and represents a major achievement in fungal phylogeny akin to the earlier large-scale collaborative molecular studies on angiosperms (e.g., Chase et al., 1993; Soltis et al., 2000). The article by Pryer et al. (2004) on fern phylogeny stands out among all of the papers in this issue by virtue of presenting the only fully integrated study of both the branching pattern and divergence times of a particular group of plants, both derived from the same set of gene sequence data. The article by Burleigh and Mathews (2004) on seed plant phylogeny establishes a seemingly robust framework tree for gymnosperms and provides important evidence for the single most controversial and revolutionary hypothesis generated from molecular analyses of any group of plants, namely, that conifers are paraphyletic, with Gnetales sister to Pinaceae to the exclusion of all other conifers. Although these three articles largely focus on new molecular data sets and phylogenetic analyses, in keeping with the nature of this issue, the authors have also broadened their remit beyond that of the usual original article, to provide a somewhat longer than normal overview of the group in question.

The six topical articles focus on selected issues that are highly relevant to reconstructing and understanding the plant tree of life. Crane et al. (2004) emphasize the importance of integrating fossil evidence with data, both morphological and molecular, from extant plants, and also stress that a comprehensive understanding of some groups (e.g., seed plants) will require a thorough reassessment of available fossils. Kellogg and Bennetzen (2004) review the structure, evolution, and, to a limited extent, phylogenetic utility of plant nuclear genomes. Linder and Rieseberg (2004) discuss the increasingly powerful molecular approaches used to dissect the role of hybridization in plant evolution. Whereas almost all articles in this issue focus on reconstructing the branching pattern (or, sometimes, the network) of plant evolutionary history, Sanderson et al. (2004) and Crepet et al. (2004) explore a relatively recent development in molecular phylogenetics, albeit one that we expect to achieve great importance, namely, the application of gene sequence data to estimate divergence times. Crepet et al. (2004) do so as part of a broader, original effort to integrate the fossil record more thoroughly with the molecular record. Sanderson et al. (2004) note that despite the limitations of methods for estimating divergence times, recent analyses are converging on similar and reasonable age estimates of angiosperms. Finally, Friedman et al. (2004) provide a timely review of a topic of rapidly growing interest in plant biology, namely, the evolution of plant development.

Although these articles do not cover every possible topic and group that are relevant to efforts to reconstruct and interpret the plant tree of life, we believe that in sum they provide sufficiently broad and deep coverage to give a vivid sense of the excitement, progress, questions, and prospects in this important and booming area of botanical research.

THE PLANT TREE OF LIFE: A 2004 ESTIMATE

Figure 1 shows our current estimate of the overall relationships of extant "plants" (defined here as all organisms con-

taining plastids). As described in the next paragraph, this is necessarily set in the broader context of overall eukaryotic phylogeny. Figure 2 shows our estimate of both phylogenetic relationships and divergence times of extant land plants. These trees are based entirely on DNA sequence data, generated and analyzed in far too many studies to cite in this brief overview (for specific citations, see the 12 taxon-oriented articles in this issue and references therein). We emphasize that these trees are an estimate of phylogeny—of evolutionary history—and, in the case of the global tree (Fig. 1), of only one dimension at that, of branching order (but not divergence time). As with all science, we can never be certain we have obtained "the truth." At the same time, however, the power of the DNA revolution makes it likely that we will soon approach relatively great certainty in estimating most or all of the branching orders of extant plant phylogeny and will gradually achieve greater and greater understanding of the time dimension as well. Importantly, as Crane et al. (2004) and Crepet et al. (2004) caution, a more complete understanding of the tree of life can only be accomplished with the integration of fossils.

As reviewed by Keeling (2004), at its deepest level, the history of plants is a history of endosymbiosis, of their birth by primary, eukaryotic/cyanobacterial endosymbiosis and of multiple secondary, tertiary, etc., eukaryotic/eukaryotic endosymbioses that have spread plastids into several other regions of the eukaryotic world. Endosymbiosis dominates our global perspective of plant evolution, as portrayed in Fig. 1. Of the five supergroups of eukaryotes tentatively identified by Keeling (2004) and mapped onto Fig. 1, two, Primoplantae and Chromoalveolates, are largely if not entirely defined by a history of endosymbiosis. The Primoplantae were by definition born of primary, cyanobacterial endosymbiosis, and the Chromalveolates were possibly born of red algal secondary symbiosis (and if not, were then repeatedly subjected to red algal symbiosis; discussed later and Fig. 1). Two of the other three supergroups of eukaryotes have also acquired plastids and evolved "plantlike" attributes through secondary symbiosis, both times via green algae endosymbionts (Fig. 1).

Although whole-organism symbiosis represents horizontal gene transfer (HGT) on the grandest scale possible, there is little reason to suspect that HGT occurs commonly enough within eukaryotic evolution to compromise significantly our prospects for recovering the metaphorical plant tree of life. In the prokaryotic world, in contrast, HGT has shaken the very concept of whether an underlying tree structure exists to recover, the metaphor of a network of gene trees being preferred by some authorities (Gogarten et al., 2002). As noted in the next section, the prevalence of HGT in the bacterial, including cyanobacterial, world may provide important limits on our ability to answer some of the deepest, most fundamental questions about the origin and earliest evolution of plants/plastids.

The view of land plant phylogeny expressed in Fig. 2 has several messages. It tells us that most of the major groups of extant land plants are monophyletic. Extant angiosperms are monophyletic, as are gymnosperms, seed plants, ferns (albeit slightly broadened to include horsetails), euphyllophytes, lycophytes, and vascular plants. Bryophytes, shown unresolved, are the conspicuous exception here, most likely being a grade that is paraphyletic to vascular plants (Shaw and Renzaglia, 2004). Figure 2 emphasizes how asymmetric the tree of extant plant life is with respect to species richness. At the extreme, *Amborella trichopoda*, a single species endemic to New Ca-
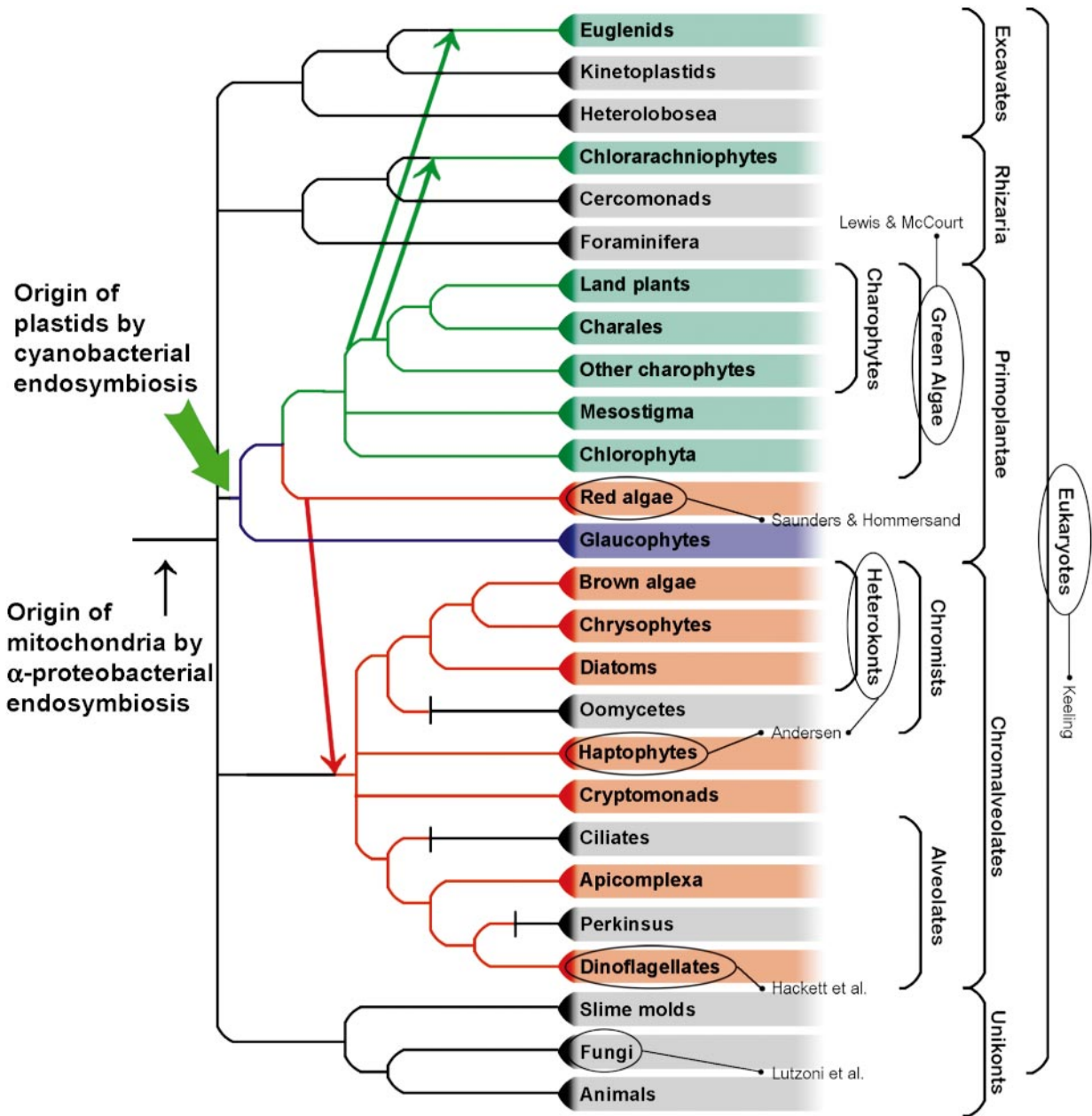
Fig. 1.   Cladogram showing phylogenetic relationships among the major groups of extant eukaryotes, with emphasis on plastid-containing groups and their evolutionary connections via primary and secondary endosymbiosis. This estimate of relationships is modified from Keeling (2004) and Baldauf et al. (2004), which should be consulted for more inclusive trees showing additional groups of nonphotosynthetic eukaryotes. The names and circumscriptions of the five eukaryotic supergroups (Excavates, etc.) are from Keeling (2004), except that we have used "Primoplantae" (J. D. Palmer, unpublished manuscript) in place of "Plants." Colors distinguish the three lineages of primary plastid-containing eukaryotes (Primoplantae) and also mark those eukaryotes with secondary plastids of red or green algal origin. The exact placement of the "symbiosis" arrows is arbitrary; essentially nothing is currently known about the timing of these events or the specific nature of the donor lineages. Two, probably independent, green algal secondary symbioses are shown, whereas the number of red algal symbioses could be as few as one (as shown) or, less likely, as many as five (see text). The three slashes indicate loss of plastids under the hypothesis of a single early red algal secondary symbiosis and Chromalveolate monophyly. "Other charophytes" denotes what is most likely a grade of four orders from which the Charales/land plant clade has arisen (Karol et al., 2001; Lewis and McCourt, 2004). Groups covered by a particular article in this special issue are circled and connected to the names of the article's authors. Branch lengths in this cladogram are entirely arbitrary; no implications with respect to time are intended.

ledonia, is probably the sister group to all other living angiosperms, numbering over 250 000 species. Similar asymmetries are evident at various places on the tree (Fig. 2), making it clear that rates of speciation and extinction vary widely over time and from group to group. The series of short internodes at the base of several major groups—angiosperms, gymnosperms, and ferns—implies relatively rapid emergence of the major lineages of each group from a common ancestor. Most of the diversity of these clades is not, however, represented on this tree. For species-rich groups such as the eudicots and
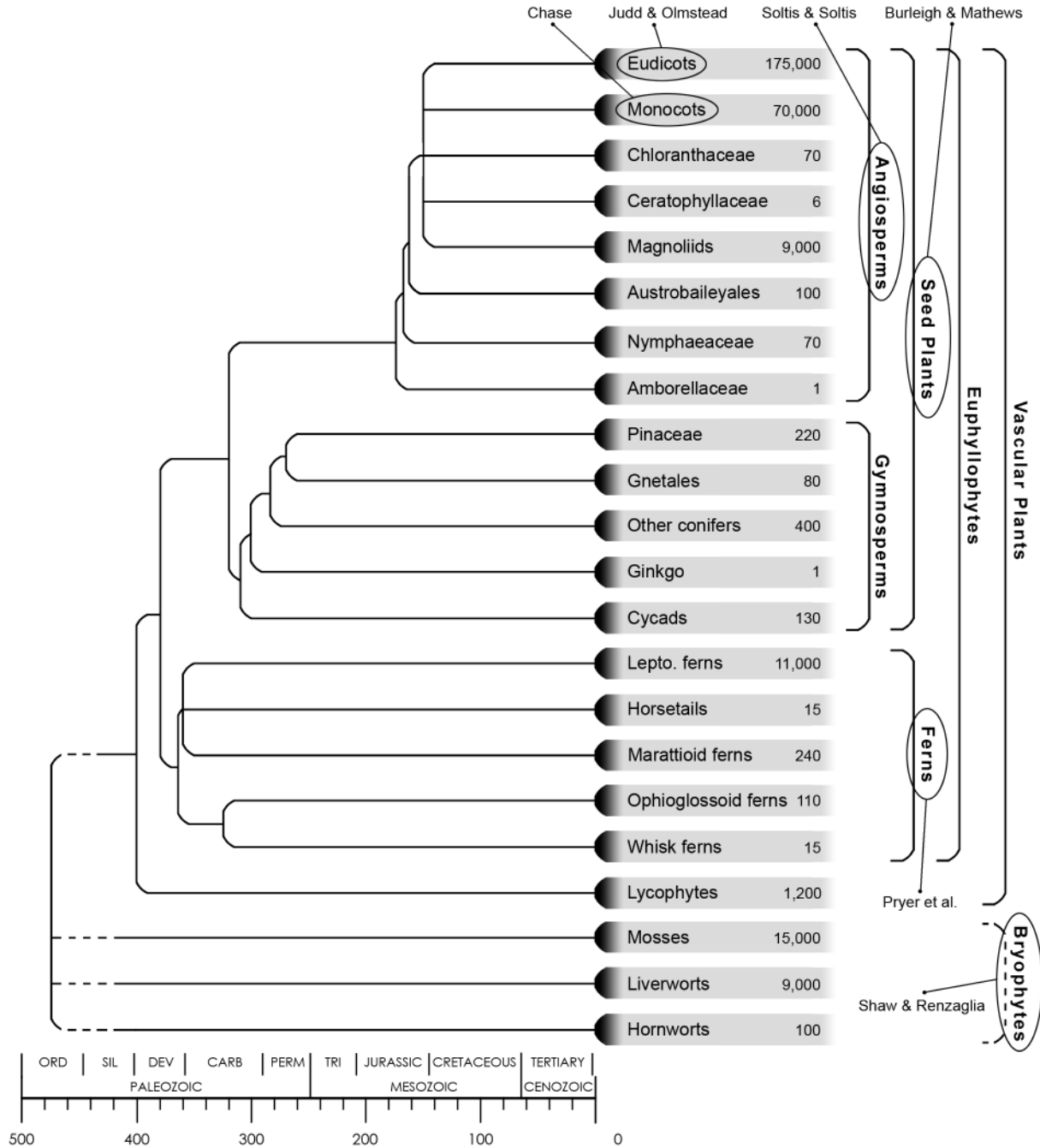
Fig. 2. Chronogram showing estimates of phylogenetic relationships and divergence times among the major groups of extant land plants. The estimate of relationships is synthesized from the following papers in this issue: Burleigh and Mathews (2004), Pryer et al. (2004), Shaw and Renzaglia (2004), and Soltis and Soltis (2004). Divergence time estimates are mostly based on analyses of molecular data with fossil constraints (Wikström et al., 2001; Pryer et al., 2004) and are augmented by fossil evidence (Kenrick and Crane, 1997; Wellman et al., 2003). Estimates of the number of species in each group are from Judd et al. (2002) and W. S. Judd (personal communication). Groups covered by a particular article in this special issue are circled and connected to the names of the article's authors. "Other conifers" refers to the clade consisting of all conifers except for Pinaceae (see Burleigh and Mathews, 2004). "Lepto. ferns" refers to leptosporangiate ferns.

monocots, we can barely imagine how many more finely spaced internodes must exist to account for the vast number of extant species in these groups. That so much progress has already been made in resolving many of these internodes (e.g., see Chase, 2004, on resolving the backbone of the monocot tree; also see Judd and Olmstead, 2004, and Soltis and Soltis, 2004) speaks volumes to the power of thousands of (nucleotide) characters, and of judicious taxon sampling, to resolve close divergences and heralds success in ultimately resolving all but the most rapid and anciently compressed of radiations as data sets head towards the tens and even hundreds of thousands of characters.

TOPOLOGICAL CONTROVERSIES IN THE PLANT TREE OF LIFE

Although the papers in this issue amply document and review the great progress made in recent years in elucidating the plant tree of life, there is of course much work still to be done. Many plant groups have barely been touched by phylogenetic inquiry, and for others little resolution has been obtained. Even in such a well-studied group as angiosperms, much important work remains, and not just at the tips of the trees where most species-level problems await resolution in this immense clade of over 250 000 species. For example, Soltis and Soltis (2004) point out a number of major groups within angiosperms for which significant resolution is currently lacking. These include not only the deep level pentachotomy shown in Fig. 2 (of eudicots, monocots, Chloranthaceae, Ceratophyllaceae, and magnoliids), but also the major groups that make up the immense (175 000 species) clade known as eudicots.

We will not discuss any further these many areas of non-controversial poor resolution. Likewise, we will not explore here any of the major methodological controversies that surround the business of inferring the plant tree of life. Some of these are, however, touched on in the following sections on case studies of topological controversy, and include such topics as the appropriate generation and use of DNA vs. other evidence in inferring phylogeny (e.g., Scotland et al., 2003), trade-offs between sampling more taxa vs. more characters (Chase et al., 1993; Soltis et al., 2004), the limits and pitfalls of using molecular data to date plant divergence times (Crepet et al., 2004; Sanderson et al., 2004), and how best to construct and assess phylogenetic trees from DNA sequence data (Felsenstein, 2004; Albert, 2005). Instead we focus on six current controversies in plant phylogeny, chosen because they all concern deep and important issues in plant evolutionary history, relate to taxa shown in Figs. 1 and 2, and illustrate a variety of biological and methodological issues in phylogeny reconstruction.

How many origins of plastids/plants?—The two deepest questions in plant phylogeny concern the birth of plastids/plants via cyanobacterial endosymbiosis: Did plastids arise once or more than once, i.e., was there but a single or as many as three cyanobacterial endosymbioses to establish the three lineages of clearly monophyletic, primary plastid-containing eukaryotes (green algae, red algae, and glaucophytes)? And relatedly, which lineage(s) of cyanobacteria gave rise to plastids? Although we have no clue of an answer to the second question, longstanding controversy over the former has subsided over the past decade. This is because DNA sequence-based trees and a number of features of plastid genomes, the plastid protein import apparatus, and photosynthetic pigment proteins have converged to provide clear (to some observers, overwhelming) evidence for a single origin of plastids (Fig. 1; Palmer, 2003; Keeling, 2004; McFadden and van Dooren, 2004), although there are still reasons (Palmer, 2003; Stiller, 2003; Stiller et al., 2003) to be cautious in assuming that this issue is completely settled. It should also be emphasized that the evidence for a common origin of plastids is much stronger for green and red algae than for the comparatively poorly studied glaucophytes (only a single glaucophyte plastid genome has been sequenced and scant information is available on glaucophyte nuclear and mitochondrial genomes). Although very rare in plastids, HGT may be sufficiently common in cyanobacteria (Zhaxbayeva et al., 2004) to bedevil attempts to ever completely settle the issue of how many primary plastid origins and, especially, to identify the cyanobacterial progenitor(s) of plastids.

What happened after plastids arose?—If plastids did indeed arise only once, then a major ensuing issue is, what is the branching order among the three lineages of Primoplantae? Keeling (2004) summarizes evidence that leads him to conclude that it "seems likely" that the glaucophytes are sister to green algae plus red algae (as shown in Fig. 1), whereas we view the issue as largely unsettled. The strongest support for this relationship among plastid genes has always come from plastid small subunit rDNA (e.g., Turner et al., 1999). However, a recent analysis of plastid large subunit rDNA, either alone or, more importantly, in combination with small subunit rDNA, finds instead strong (99% bootstrap) support for green algae as sister to red algae plus glaucophytes (S. Turner, K. M. Pryer, and J. D. Palmer, unpublished data). Although most analyses of very large character sets (of ca. 40 plastid protein genes) find strong support for glaucophytes being "deepest" (e.g., Martin et al., 2002), at least one is essentially unresolved on this issue (Turmel et al., 1999). Critically, all such analyses are plagued by woefully inadequate taxon sampling (only a single glaucophyte, a single cyanobacterial outgroup, and just a few each of red and green algae), which, especially at this level of divergence, raises the specter of obtaining strong support for an incorrect topology (Soltis et al., 2004). Although the only relevant nuclear multigene analysis appears to lack resolution on this issue (Moreira et al., 2000), red and green algae do share a nuclear gene duplication/plastid targeting event to the exclusion of glaucophytes (Rogers and Keeling, 2003).

Many more sequence data (especially from glaucophytes) are clearly needed to resolve this key issue, which greatly affects interpretation of various important aspects of plastid evolution, such as their history of gene loss (Martin et al., 2002) and cell wall loss. For example, glaucophytes uniquely retain a bacterial-like, peptidoglycan cell wall around their plastids: Did green and red plastids lose their cell wall independently or in a common ancestor? Unfortunately, the apparent predilection of cyanobacteria (the only good outgroups for this inquiry) to engage in HGT (Zhaxbayeva et al., 2004) compromises, perhaps severely or even irrevocably, our ability to answer this question.

How many secondary symbioses of red algae?—Perhaps the most important question relating to the symbiotic history of plastids concerns the number of red algal secondary symbioses. The diversity of eukaryotes with secondary red plastids is immense, and these are phylogenetically interspersed with groups that lack plastids (Fig. 1). This has led many observers to postulate multiple secondary symbioses involving a red algal endosymbiont. As reviewed by Keeling (2004), however, recent DNA evidence, both sequence-based and rearrangement-based, now provides reason to think that all secondary red algal plastids trace back to a common secondary symbiosis (Fig. 1, red arrow), in the common ancestor of a putative eukaryotic supergroup—Chromalveolates—first postulated by Cavalier-Smith (1998). If Chromalveolates are indeed monophyletic, arising by only a single red algae symbiosis, then this means that the many diverse lineages within the group that apparently lack plastids (see Keeling, 2004, for many such

examples beyond the three shown in Fig. 1) must have once had them. In the case of ciliates and oomycetes (Fig. 1), initial analysis of complete nuclear genomes has failed to find any significant vestiges of putative plastid-derived genes (P. J. Keeling, University of British Columbia, personal communication). Either ciliates and oomycetes have been cleansed of their plastid heritage to an extent unthinkable for land plants (Martin et al., 2002) or else the evidence for Chromalveolate monophyly and a single red algal secondary symbiosis is misleading. Clearly, much more phylogenetic and genomic evidence is needed.

Where does Mesostigma belong?—As reviewed by Lewis and McCourt (2004), a major puzzle in green algal phylogeny concerns the placement of *Mesostigma*, an unusual asymmetrical unicell. With good taxon sampling, a four-gene, three-genome data set strongly supports *Mesostigma* within charophytes, probably as sister to the rest of this clade (Karol et al., 2001), whereas a two-gene plastid data set places it with equally strong support as sister to all green algae, prior to what is otherwise regarded as the fundamental split in green algal evolution (between charophytes and chlorophytes; Turmel et al., 2002a; Fig. 1). With poor taxon sampling, a similar conflict arises for much larger character sets. Some analyses of combined matrices of ca. 40 plastid genes place *Mesostigma* within the charophytes (e.g., Martin et al., 2002), whereas other such analyses and those of 19 mitochondrial genes place it as sister to all other green algae (Lemieux et al., 2000; Turmel et al., 2002b). As with many critical phylogenetic issues in plants and other organisms, robust resolution of the position of *Mesostigma* may require both large character sets and better taxon sampling. Resolving the placement of *Mesostigma* is important to understanding the early evolution of green algae, as well as evolutionary trends in organellar gene content (Lemieux et al., 2000; Turmel et al., 2002b) and photosynthetic pigments (Yoshii et al., 2003).

Who's at the base of land plants?—A major controversy in land plant phylogeny concerns the base of the tree (Fig. 2). Traditionally, land plants have been divided into two groups, vascular plants and bryophytes. Although vascular plants are strongly supported as monophyletic based on both DNA evidence (e.g., Nickrent et al., 2000) and morphology (Kenrick and Crane, 1997), bryophytes are now generally thought to comprise a grade of three monophyletic lineages (mosses, liverworts, and hornworts) of uncertain relationship to each other and to vascular plants. Many studies (listed in Shaw and Renzaglia, 2004) have addressed these relationships. In our view there is as yet no clear answer, and therefore we show the base of land plants as a tetrachotomy (Fig. 2). Most commonly, either liverworts or hornworts emerge as sister to all land plants, but we see little reason to favor one result over the other based on current data. Those who do, e.g., favoring liverworts-sister on the basis of "compelling evidence" (Friedman et al., 2004) from so-called "rare genomic markers" (mitochondrial intron presence/absence; Qiu et al., 1998; Dombrovska and Qiu, 2004) are in our view privileging a select few (not so rarely changing) characters over thousands of other characters for which we have a long history of robust use. Such purportedly rare events can be subject to high levels of homoplasy when examined with intensive sampling (Adams et al., 2002; McPherson et al., 2004), and thus we do not really know how reliable any of these sorts of characters are.

Figure 2 shows a number of surprising placements and relationships that have emerged solely from and/or found strong support only from DNA sequences. Within ferns, these include the placement of horsetails within the ferns and the sister-group relationship of the enigmatic, vegetatively reduced Psilotales and the ophioglossoid ferns (Pryer et al., 2004). Within angiosperms, we note the deep positions of the monotypic, New Calendonian-endemic, Amborellaceae, and of Nymphaeaceae and Austrobaileyales (Soltis and Soltis, 2004). Also not shown are the many other surprising placements that have emerged within groups of land plants (and also nonland plants) not covered by the obviously scanty representation afforded by these two global, framework trees. In almost all these cases, however, the surprise has been one of delight ("Ah ha, we've finally found a place for that troublesome species X.") rather than one of dismay or disbelief ("There's no way species Y belongs in *that* group; something has got to be wrong with these DNA data."). Frequently, careful assessment of morphology reveals characters that agree with the "DNA surprise," although in many instances these characters may be cryptic (Judd and Olmstead, 2004).

Are Gnetales really sister to Pinaceae?—The most radical, shocking, and controversial placement of any group of plants concerns Gnetales, a small, relatively obscure group of gymnosperms. Whereas a notable hypothesis from morphological cladistic studies had been that Gnetales were sister to angiosperms, many molecular studies instead found gymnosperms to be monophyletic and placed Gnetales *within* conifers, as sister to Pinaceae (e.g., Bowe et al., 2000; Chaw et al., 2000; reviewed in Burleigh and Mathews, 2004). Although other molecular studies found support for monophyly of conifers and instead placed Gnetales either as sister to conifers or as sister to all other seed plants, the original analyses in the paper by Burleigh and Mathews (2004) in this issue provide strong evidence for the "gnepines" hypothesis (Bowe et al., 2000; Chaw et al., 2000), i.e., that Gnetales and Pinaceae are sister taxa. Burleigh and Mathews (2004) sampled moderately extensively within gymnosperms and examined more genes (13 total; five plastid, four nuclear, and four mitochondrial) and characters (almost 19 000 nucleotides) than in any other previous study on this issue. Contrary to previous claims (e.g., Rydin et al., 2002) that earlier, three-genome analyses favoring the gnepines topology rested "almost exclusively" on mitochondrial genes and that there is a "severe conflict between the mitochondrial and other genomes [that] should not be suppressed or ignored," Burleigh and Mathews (2004) found that all three genomic data partitions support the gnepines result. This was true in all maximum likelihood analyses and in those parsimony analyses in which the most rapidly evolving sites were excluded. Indeed, contra Rydin et al. (2002), the mitochondrial partition actually provided only slightly lower bootstrap support for gnepines in these analyses than did the nuclear or plastid partitions. Moreover, when all 13 genes were combined (and with the fastest sites excluded for parsimony), both parsimony and maximum likelihood gave 100% bootstrap support for gnepines.

Only in parsimony analyses that included all sites did Burleigh and Mathews (2004) fail to recover the gnepines topology. Tellingly, however, these analyses (with all 13 genes) placed Gnetales in a radically different position, as sister to all other seed plants, prompting Burleigh and Mathews (2004) to conclude that the "Gnetales-sister signal is restricted to sites

[largely in plastid genes] in the fastest two of nine evolutionary rate categories" and reflects bias "in the most rapidly evolving sites to which parsimony is particularly sensitive." In short, the Gnetales-sister placement is a likely example of long-branch-attraction (between the long branches leading to Gnetales and to the outgroups), and the gnepines topology is likely to be correct. Nonetheless, we also caution that this issue should not be considered settled; even though receiving 100% bootstrap support in most 13-gene analyses, the gnepines topology is, as Burleigh and Mathews (2004) point out, "very similar" to the gnetifer topology (Gnetales sister to a monophyletic conifers) "and potentially even a small amount of error or bias could influence [these] phylogenetic results."

We emphasize this particular case because it nicely illustrates a variety of issues in phylogenetics. It illustrates the potential to get artifactual results (e.g., the Gnetales-sister placement) under the following evolutionary conditions: (1) high levels of extinction (on, among others, the outgroup branch and the branches leading to Gnetales, angiosperms, and *Ginkgo*), (2) high rates of sequence evolution (in Gnetales), and (3) biased evolution at a subset of sites (rapidly evolving sites in plastid and nuclear genes). The first two of these factors—extensive extinction and rapid sequence evolution—are probably the greatest intrinsic problems in molecular phylogenetics, especially when operating in concert, as with the Gnetales placement. This case vividly illustrates how different partitions of a sequence data set can generate highly supported trees with radically different topologies and also points to an apparently useful methodological approach to deal with this intragenic conflict. It also illustrates the superior performance of maximum likelihood over parsimony under these challenging conditions (whereas parsimony generally performs well in situations where extinction has not eliminated the potential for extensive taxon sampling to break up the long branches that are particularly problematic for it). Finally, it shows the potential for morphology to be difficult to interpret and even unhelpful in phylogenetic inference. At the same time, it is important to note that the gnepines topology conflicts with relatively few morphological characters, especially ones that provide a clear pattern (Burleigh and Mathews, 2004; Soltis et al., in press). This points to the dangerously seductive influence that "charismatic singular characters," be they morphological (Scotland et al., 2003) or "rare genomic structural" (McPherson et al., 2004), can exert compared to the numerically overwhelming but unlovable mass of nucleotide sequence characters that are the foundation of virtually all well-supported phylogenetic trees.

PROSPECTS

With all the impressive progress made over the first 20 years or so of the DNA revolution in plant systematics, it is important to recognize that the revolution is still at an early stage. Few land plants, and even fewer algae, have been sampled for even one gene, and fewer still for even two to four genes. Even though great progress has been made in working out the overall framework tree of angiosperms—the most intensively studied group of plants and one that also offers the benefits of being relatively young and species rich (low rates of extinction and/or high rates of speciation minimize long-branch-attraction, which is probably the greatest overall bugaboo of DNA systematics)—many important issues remain to be worked out

(Chase, 2004; Judd and Olmstead, 2004; Soltis and Soltis, 2004).

The next 20 years will undoubtedly see an ever more rapid accumulation of DNA sequence data and the generation of comprehensive, robustly resolved, and increasingly well-dated phylogenetic trees. Phylogenetics is now hitched to a powerful engine—the economic, largely biomedical engine that has been driving the development of faster and cheaper technologies for sequencing DNA and for high-throughput robotic handling of DNA in general. Even at the current rate of data accumulation, nearly all of the remaining problems in plants are tractable, and it is likely that there will continue to be improvements that will increase output and hasten progress. The next 20 years may even see radical breakthroughs in sequencing and other DNA technologies, breakthroughs that could allow the virtually limitless collection of gene and even whole genome data from most plants (especially from the small but gene-rich genome of plastids). If so, then we will certainly look back on this era as a small waystation on the path toward reconstructing the complex and fascinating evolutionary history of the botanical world.

The prospects for achieving a robustly resolved and well-dated tree of plant life are exhilarating. To rephrase Dobzhansky (1973), "Nothing in biology makes sense except in light of phylogeny." Only with an accurate tree in hand can we properly make sense of the profound richness and diversity of the botanical world. Only then can we fully appreciate evolution at all levels of organization, from the ecological and morphological to the biochemical and genomic. Specific applications of phylogenetic studies, of "tree thinking," are too numerous to list here, ranging from the applied (Yates et al., 2004) to fundamental studies of evolutionary and ecological processes (36 such applications are listed in Table 3.1 of Futuyma, 2004). Hillis (2004) aptly argues that "*as the Tree of Life becomes more complete, its applications are also expanding exponentially. A complete Tree of Life would allow analyses that we would never contemplate today.*" He also contends that before long the phylogenetic revolution will have permeated the way we study all areas of biology. The study of the plant tree of life has, through the collaborative spirit of the field and its remarkable progress, set the standard for tree of life efforts, and we look forward to accelerating rates of progress in the years to come.

LITERATURE CITED

ADAMS, K. L., Y.-L. QIU, M. STOUTEMYER, AND J. D. PALMER. 2002. Punctuated evolution of mitochondrial gene content: high and variable rates of mitochondrial gene loss and transfer during angiosperm evolution. *Proceedings of the National Academy of Sciences, USA* 99: 9905–9912.

ALBERT, V. A. [ED.]. 2005. Parsimony, phylogeny, and genomics. Oxford Press, Oxford, UK, in press.

ANDERSEN, R. A. 2004. Biology and systematics of heterokont and haptophyte algae. *American Journal of Botany* 91: 1508–1522.

BALDAUF, S. L., D. BHATTACHARYA, J. COCKRILL, P. HUGENHOLTZ, J. PAWLOWSKI, AND A. G. B. SIMPSON. 2004. The tree of life: an overview. *In* J. Cracraft and M. J. Donoghue [eds.], Assembling the tree of life, 43–75. Oxford University Press, Oxford, UK.

BOWE, L. M., G. COAT, AND C. W. DE PAMPHILIS. 2000. Phylogeny of seed plants based on all three genomic compartments: extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. *Proceedings of the National Academy, USA* 97: 4092–4097.

BURLEIGH, J. G., AND S. MATHEWS. 2004. Phylogenetic signal in nucleotide data from seed plants: implications for resolving the seed plant tree of life. *American Journal of Botany* 91: 1599–1613.

CAVALIER-SMITH, T. 1998. A revised six-kingdom system of life. *Biological Reviews of the Cambridge Philosophical Society* 73: 203–266.

CHASE, M. W. 2004. Monocot relationships: an overview. *American Journal of Botany* 91: 1645–1655.

CHASE, M. W., D. E. SOLTIS, R. G. OLMSTEAD, D. MORGAN, D. H. LES, B. D. MISHLER, M. R. DUVALL, R. A. PRICE, H. G. HILLS, Y.-L. QIU, K. A. KRON, J. H. RETTIG, E. CONTI, J. D. PALMER, J. R. MANHART, K. J. SYTSMA, H. J. MICHAELS, W. J. KRESS, K. G. KAROL, D. CLARK, M. HEDREN, B. S. GAUT, R. K. JANSEN, K.-J. KIM, C. F. WIMPEE, J. F. SMITH, G. R. FURNIER, S. H. STRAUSS, Q.-Y. XIANG, G. M. PLUNKETT, P. S. SOLTIS, S. M. SWENSEN, S. E. WILLIAMS, P. A. GADEK, C. J. QUINN, L. E. EGUIARTE, E. GOLENBERG, G. H. LEARN, JR., S. W. GRAHAM, S. C. H. BARRETT, S. DAYANANDAN, AND V. A. ALBERT. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 528–580.

CHAW, S. M., C. L. PARKINSON, Y. C. CHENG, T. M. VINCENT, AND J. D. PALMER. 2000. Seed plant phylogeny inferred from all three plant genomes: monophyly of extant gymnosperms and origin of Gnetales from conifers. *Proceedings of the National Academy, USA* 97: 4086–4091.

CRANE, P. R., P. S. HERENDEEN, AND E. M. FRIIS. 2004. Fossils and plant phylogeny. *American Journal of Botany* 91: 1683–1699.

CREPET, W. L., K. C. NIXON, AND M. A. GANDOLFO. 2004. Fossil evidence and phylogeny: the age of major angiosperm clades based on mesofossil and macrofossil evidence from Cretaceous deposits. *American Journal of Botany* 91: 1666–1682.

DOBZHANSKY, T. 1973. Nothing in biology makes sense except in the light of evolution. *The American Biology Teacher* 35: 125–129.

DOMBROVSKA, O., AND Y.-L. QIU. 2004. Distribution of introns in the mitochondrial gene *nad1* in land plants: phylogenetic and molecular evolutionary implications. *Molecular Phylogenetics and Evolution* 32: 246–263.

FELSENSTEIN, J. 2004. Inferring phylogenies. Sinauer, Sunderland, Massachusetts, USA.

FRIEDMAN, W. E., R. C. MOORE, AND M. D. PURUGGANAN. 2004. The evolution of plant development. *American Journal of Botany* 91: 1726–1741.

FUTUYMA, D. J. 2004. The fruit of the tree of life: insights into evolution and ecology. *In* J. Cracraft and M. J. Donoghue [eds.], Assembling the Tree of Life, 25–39. Oxford University Press, Oxford, UK.

GOGARTEN, J. P., W. F. DOOLITTLE, AND J. G. LAWRENCE. 2002. Prokaryotic evolution in light of gene transfer. *Molecular Biology and Evolution* 19: 2226–2238.

HACKETT, J. D., D. M. ANDERSON, D. L. ERDNER, AND D. BHATTACHARYA. 2004. Dinoflagellates: a remarkable evolutionary experiment. *American Journal of Botany* 91: 1523–1534.

HILLIS, D. M. 2004. The tree of life and the grand synthesis of biology. *In* J. Cracraft and M. J. Donoghue [eds.], Assembling the tree of life, 545–547. Oxford University Press, Oxford, UK.

JUDD, W. S., AND R. G. OLMSTEAD. 2004. A survey of tricolpate (eudicot) diversity. *American Journal of Botany* 91: 1627–1644.

JUDD, W. S., C. S. CAMPBELL, E. A. KELLOGG, P. F. STEVENS, AND M. J. DONOGHUE. 2002. Plant systematics: a phylogenetic approach. Sinauer, Sunderland, Massachusetts, USA.

KAROL, K. G., R. M. MCCOURT, M. T. CIMINO, AND C. F. DELWICHE. 2001. The closest living relatives of land plants. *Science* 294: 2351–2353.

KEELING, P. J. 2004. Diversity and evolutionary history of plastids and their hosts. *American Journal of Botany* 91: 1481–1493.

KELLOGG, E. A., AND J. L. BENNETZEN. 2004. The evolution of nuclear genome structure in plants. *American Journal of Botany* 91: 1481–1493.

KENRICK, P., AND P. R. CRANE. 1997. The origin and early diversification of land plants: a cladistic study. Smithsonian Institution, Washington, D.C., USA.

LEMIEUX, C., C. OTIS, AND M. TURMEL. 2000. Ancestral chloroplast genome in *Mesostigma viride* reveals an early branch of green plant evolution. *Nature* 403: 649–652.

LEWIS, L. A., AND R. M. MCCOURT. 2004. Green algae and the origin of land plants. *American Journal of Botany* 91: 1535–1556.

LINDER, C. R., AND L. H. RIESEBERG. 2004. Reconstructing patterns of reticulate evolution in plants. *American Journal of Botany* 91: 1535–1556.

LUTZONI, F., F. KAUFF, C. J. COX, D. MCLAUGHLIN, G. CELIO, B. DENTINGER, M. PADAMSEE, D. HIBBETT, T. Y. JAMES, E. BALOCH, M. GRUBE, V. REEB, V. HOFSTETTER, C. SCHOCH, A. E. ARNOLD, J. MIADLIKOWSKA, J. SPATAFORA, D. JOHNSON, S. HAMBLETON, M. CROCKETT, R. SHOEMAKER, G.-H. SUNG, R. LÜCKING, T. LUMBSCH, K. O'DONNELL, M.

BINDER, P. DIEDERICH, D. ERTZ, C. GUEIDAN, B. HALL, K. HANSEN, R. C. HARRIS, K. HOSAKA, Y.-W. LIM, Y. LIU, B. MATHENY, H. NISHIDA, D. PFISTER, J. ROGERS, A. ROSSMAN, I. SCHMITT, H. SIPMAN, J. STONE, J. SUGIYAMA, R. YAHR, AND R. VILGALYS. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* 91: 1446–1480.

MARTIN, W., T. RUJAN, E. RICHLY, A. HANSEN, S. CORNELSEN, T. LINS, D. LEISTER, B. STOEBE, M. HASEGAWA, AND D. PENNY. 2002. Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proceedings of the National Academy of Sciences, USA* 99: 12246–12251.

MCFADDEN, G. I., AND G. G. VAN DOOREN. 2004. Evolution: Red algal genome confirms common origin of all plastids. *Current Biology* 14: R514–R516.

MCPHERSON, M. A., M. F. FAY, M. W. CHASE, AND S. W. GRAHAM. 2004. Parallel loss of a slowly evolving intron from two closely related families in Asparagales. *Systematic Botany* 29: 296–307.

MOREIRA, D., H. LE GUYADER, AND H. PHILLIPPE. 2000. The origin of red algae and the evolution of chloroplasts. *Nature* 405: 69–72.

NICKRENT, D. L., C. L. PARKINSON, J. D. PALMER, AND R. J. DUFF. 2000. Multigene phylogeny of land plants with special reference to bryophytes and the earliest land plants. *Molecular Biology and Evolution* 17: 1885–1895.

PALMER, J. D. 2003. The symbiotic birth and spread of plastids: how many times and whodunit? *Journal of Phycology* 39: 1–9.

PRYER, K. M., E. SCHUETTPELZ, P. G. WOLF, H. SCHNEIDER, A. R. SMITH, AND R. CRANFILL. 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on early leptosporangiate lineages. *American Journal of Botany* 91: 1582–1598.

QIU, Y.-L., Y. R. CHO, J. C. COX, AND J. D. PALMER. 1998. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. *Nature* 394: 671–674.

ROGERS, M. B., AND P. J. KEELING. 2003. Lateral gene transfer and re-compartmentalisation of Calvin cycle enzymes in plants and algae. *Journal of Molecular Evolution* 58: 367–375.

RYDIN, C., M. KÄLLERSJÖ, AND E. M. FRIIS. 2002. Seed plant relationships and the systematic position of Gnetales based on nuclear and chloroplast DNA: conflicting data, rooting problems and the monophyly of conifers. *International Journal of Plant Sciences* 163: 197–214.

SANDERSON, M. J., J. L. THORNE, N. WIKSTRÖM, AND K. G. BREMER. 2004. Molecular evidence on plant divergence times. *American Journal of Botany* 91: 1656–1665.

SAUNDERS, G. W., AND M. H. HOMMERSAND. 2004. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. *American Journal of Botany* 91: 1494–1507.

SCOTLAND, R. W., R. G. OLMSTEAD, AND J. R. BENNETT. 2003. Phylogeny reconstruction: the role of morphology. *Systematic Biology* 52: 539–548.

SHAW, A. J., AND K. S. RENZAGLIA. 2004. The phylogeny and diversification of bryophytes. *American Journal of Botany* 91: 1557–1581.

SOLTIS, P. S., AND D. E. SOLTIS. 2004. The origin and diversification of angiosperms. *American Journal of Botany* 91: 1614–1626.

SOLTIS, D. E., P. S. SOLTIS, M. W. CHASE, M. E. MORT, D. C. ALBACH, M. ZANIS, V. SAVOLAINEN, W. J. HAHN, S. B. HOOT, M. F. FAY, M. AXTELL, S. M. SWENSEN, L. M. PRINCE, W. J. KRESS, K. C. NIXON, AND J. S. FARRIS. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL,* and *atpB* sequences. *Botanical Journal of the Linnean Society* 133: 381–461.

SOLTIS, D. E., V. A. ALBERT, V. SAVOLAINEN, K. HILU, Y.-L. QIU., M. W. CHASE, J. S. FARRIS, S. STEFANOVIĆ, D. W. RICE, J. D. PALMER, AND P. S. SOLTIS. 2004. Genome-scale data, angiosperm relationships, and ending incongruence: a cautionary tale in phylogenetics. *Trends in Plant Science* 9.

SOLTIS, D. E., P. S. SOLTIS, P. K. ENDRESS, AND M. W. CHASE. In press. Phylogeny and evolution of angiosperms. Smithsonian Institution Press, Washington, D.C., USA.

STILLER, J. W. 2003. Weighing the evidence for a single origin of plastids. *Journal of Phycology* 39: 1283–1285.

STILLER, J. W., D. C. REEL, AND J. C. JOHNSON. 2003. A single origin of plastids revisited: convergent evolution in organelle genome content. *Journal of Phycology* 39: 95–105.

TURMEL, M., C. OTIS, AND C. LEMIEUX. 1999. The complete chloroplast DNA sequence of the green alga *Nephroselmis olivacea*: insights into

the architecture of ancestral chloroplast genomes. *Proceedings of the National Academy of Sciences, USA* 96: 10248–10253.

TURMEL, M., M. EHARA, C. OTIS, AND C. LEMIEUX. 2002a. Phylogenetic relationships among streptophytes as inferred from chloroplast small and large subunit rRNA gene sequences. *Journal of Phycology* 38: 364–375.

TURMEL, M., C. OTIS, AND C. LEMIEUX. 2002b. The complete mitochondrial DNA sequence of *Mesostigma viride* identifies this green alga as the earliest green plant divergence and predicts a highly compact mitochondrial genome in the ancestor of all green plants. *Molecular Biology and Evolution* 19: 24–38.

TURNER, S., K. M. PRYER, V. P. MIAO, AND J. D. PALMER. 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *Journal of Eukaryotic Microbiology* 46: 327–338.

WELLMAN, C. H., P. L. OSTERLOFF, AND U. MOHIUDDIN. 2003. Fragments of the earliest land plants. *Nature* 425: 282–285.

WIKSTRÖM, N., V. SAVOLAINEN, AND M. W. CHASE. 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London, Series B* 268: 2211–2220.

YATES, T. L., J. SALAZAR-BRAVO, AND J. W. DRAGOO. 2004. The importance of the tree of life to society. *In* J. Cracraft and M. J. Donoghue [eds.], Assembling the tree of life, 7–17. Oxford University Press, Oxford, UK.

YOSHII, Y., S. TAKAICHI, T. MAOKA, AND I. INOUYE. 2003. Photosynthetic pigment composition in the primitive green alga *Mesostigma viride* (Prasinophyceae): phylogenetic and evolutionary implications. *Journal of Phycology* 39: 570–576.

ZHAXYBAYEVA, O., P. LAPIERRE, AND J. P. GOGARTEN. 2004. Genome mosaicism and organismal lineages. *Trends in Genetics* 20: 254–260.