

GUNNERALES ARE SISTER TO OTHER CORE EUDICOTS: IMPLICATIONS FOR THE EVOLUTION OF PENTAMERY¹

DOUGLAS E. SOLTIS,^{2,8} ANNE E. SENTERS,³ MICHAEL J. ZANIS,³
SANGTAE KIM,² JAMES D. THOMPSON,² PAMELA S. SOLTIS,⁴
LOUIS P. RONSE DE CRAENE,⁵ PETER K. ENDRESS,⁶ AND
JAMES S. FARRIS⁷

²Department of Botany and the Genetics Institute, University of Florida, Gainesville, Florida 32611 USA;

³School of Biological Sciences, Washington State University, Pullman, Washington 99164 USA;

⁴Florida Museum of Natural History and the Genetics Institute, University of Florida, Gainesville, Florida 32611 USA;

⁵Royal Botanic Gardens Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, Scotland, UK;

⁶Institute of Systematic Botany, University of Zurich, 8008 Zurich, Switzerland; and

⁷Molekylärsystematiska Laboratoriet, Naturhistoriska Riksmuseet, Box 50007, S 104 05 Stockholm, Sweden

Phylogenetic relationships among many lineages of angiosperms have been clarified via the analysis of large molecular data sets. However, with a data set of three genes (18S rDNA, *rbcL*, and *atpB*), relationships among lineages of core eudicots (Berberidopsidales, Caryophyllales, Gunnerales, Santalales, Saxifragales, asterids, rosids) remain essentially unresolved. We added 26S rDNA sequences to a three-gene matrix for 201 eudicots (8430 base pair aligned nucleotides per taxon). Parsimony analyses provided moderate (84%) jackknife support for Gunnerales, which comprise the two enigmatic families Gunneraceae and Myrothamnaceae, as sister to all other core eudicots. This position of Gunnerales has important implications for floral evolution. A dimerous or trimerous perianth is frequently encountered in early-diverging eudicots (e.g., Buxaceae, Proteales, Ranunculales, Trochodendraceae), whereas in core eudicots, pentamery predominates. Significantly, dimery is found in Gunneraceae and perhaps Myrothamnaceae (the merosity of the latter has also been interpreted as labile). Parsimony reconstructions of perianth merosity demonstrate lability among early-diverging eudicots and further indicate that a dimerous perianth could be the immediate precursor to the pentamerous condition characteristic of core eudicots. Thus, the developmental canalization that yielded the pentamerous condition of core eudicots occurred after the node leading to Gunnerales.

Key words: eudicot; Gunnerales; molecular phylogeny; perianth merosity.

Enormous strides have been made in understanding relationships among angiosperms, in part because of the phylogenetic analysis of large data sets involving numerous taxa and the combination of several genes (e.g., Hoot et al., 1999; Qiu et al., 1999; Soltis et al., 1999, 2000; Savolainen et al., 2000a, b). These and other studies have revealed the major clades of angiosperms and clarified some of the most vexing problems in angiosperm systematics, including the root of the angiosperms (e.g., Mathews and Donoghue, 1999; Parkinson et al., 1999; Qiu et al., 1999, 2000; Barkman et al., 2000; Bowe et al., 2000; Graham and Olmstead, 2000; Zanis et al., 2002) and relationships among basal angiosperms (Qiu et al., 1999; Zanis et al., 2002). Progress has also been made in elucidating relationships within some of the major clades of angiosperms, such as the monocots (Chase et al., 2000), the large asterid clade (e.g., Olmstead et al., 2000; Albach et al., 2001), Santalales (Nickrent and Malécot, 2001), and Saxifragales (Fishbein et al., 2001). As a result of the great progress in clarifying phylogenetic relationships, the angiosperms became the first major group of organisms to be reclassified based on DNA sequence data (APG, 1998; APG II, 2002).

Despite the progress in elucidating deep-level phylogenetic relationships in angiosperms, major questions remain, particularly within the large eudicot clade, which contains approx-

imately 75% of all angiosperm species (Drinnan et al., 1994). The three-gene (*rbcL*, *atpB*, 18S rDNA) topology (Soltis et al., 1999, 2000), still the most comprehensive view of the angiosperms as a whole, demonstrates the monophyly of the eudicots with a high degree of confidence (100%; see also Soltis et al., 1998; Hoot et al., 1999; Savolainen et al., 2000a). Within the eudicot clade is a basal grade (Fig. 1): Ranunculales, Proteales, Sabiaceae, Buxaceae (sensu APGII, which corresponds to Didymelaceae + Buxaceae), and Trochodendraceae (including *Tetracentron*) (see also Chase et al., 1993; Soltis et al., 1997; Qiu et al., 1998; Savolainen et al., 2000a, b).

The early-diverging eudicot lineages are followed by a large, strongly supported clade referred to as the core eudicots, based on data sets of two or three genes (Hoot et al., 1999; Soltis et al., 1999, 2000; Savolainen et al., 2000a). For example, with *atpB* + *rbcL*, support for the monophyly of the core eudicots was 91%. Even in analyses involving *rbcL* alone, weak jackknife support (58%) was apparent for the core eudicot clade (Chase and Albert, 1998; Savolainen et al., 2000a, b). The core eudicot clade comprises seven subclades (Soltis et al., 2000): (1) Berberidopsidales (composed of only Berberidopsidaceae and Aextoxicaceae); (2) Gunnerales (Myrothamnaceae and Gunneraceae); (3) Saxifragales; (4) rosids; (5) Santalales; (6) Dilleniaceae + Caryophyllales; and (7) asterids (Fig. 1). The sister-group relationship of Dilleniaceae and Caryophyllales is only weakly supported; otherwise, each of these subclades is strongly supported in analyses involving two or three genes. However, relationships among these clades remain unresolved in analyses based on three genes. The only reso-

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⁸ Author for reprint requests (e-mail: dsoltis@botany.ufl.edu).

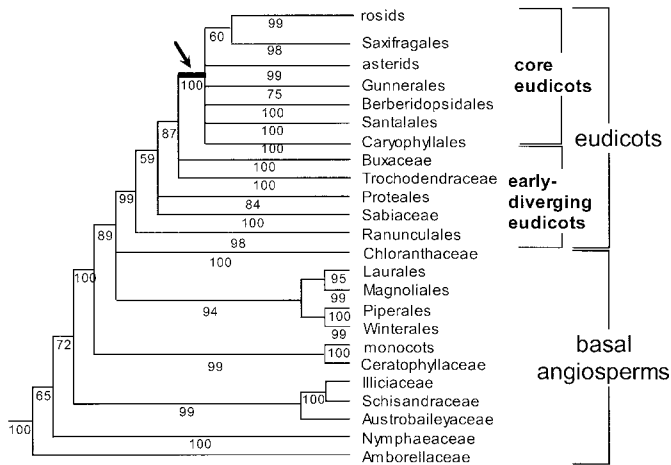


Fig. 1. Summary tree depicting our current understanding of angiosperm relationships. Relationships among eudicots are based on the three-gene topology (Soltis et al., 1999, 2000). Relationships among basal angiosperms reflect the results of Qiu et al. (1999) and Zanis et al. (2002). Numbers indicate jackknife support for clades. Arrow and bold line indicate the branch to the core eudicot clade.

lution among these seven lineages was the weakly supported (60%) Saxifragales + rosid sister group. This lack of resolution is an important limitation for both systematists and evolutionary biologists, compromising the reconstruction of character evolution on a broad scale across the eudicots.

Earlier workers (Endress, 1987a, b, 1990, 1994; Drinnan et al., 1994) considered various floral characters, including merosity and the arrangement of floral parts, to be highly labile in early-diverging eudicots. Different merosities of the perianth coexist in the basal angiosperms and early-diverging eudicots, with a predominance of trimery and dimery. On the contrary, the majority of core eudicots appear to have a stable pentamerous merosity. Several hypotheses have been put forward for the derivation and relationships of different merosities (e.g., Erbar and Leins, 1981, 1994; Endress, 1987a; Kubitzki, 1987; Ronse De Craene and Smets, 1994). However, these hypotheses have not been examined in an explicitly phylogenetic context by mapping character states onto phylogenetic trees. The mapping of floral traits by Albert et al. (1998) and Zanis et al. (in press) provided some support for the hypothesized floral lability of early-diverging eudicots, but these studies focused on basal angiosperms.

Our first objective was to improve the understanding of relationships among major lineages of core eudicots. To accomplish this goal, we added entire sequences for a fourth gene, 26S rDNA, to the existing database of sequences already available for 18S rDNA, *atpB*, and *rbcl*. The entire 26S rDNA is greater than 3000 bp, and the phylogenetic utility of both entire and partial 26S rDNA sequences has been previously noted (e.g., Kuzoff et al., 1998; Fishbein et al., 2001; Neyland, 2001; Soltis et al., 2001). Our second main objective was to reconstruct ancestral eudicot floral features (with a focus on merosity) using the four-gene topology that we obtained.

MATERIALS AND METHODS

Data sets—A data set of 206 taxa was constructed for phylogenetic analysis; 201 species represented the major lineages of eudicots, and five basal angiosperms (*Amborella*, *Cabomba*, *Magnolia*, *Chloranthus*, and *Hedyos-*

num) were used as outgroups. Sequences of 18S rDNA, *rbcl*, and *atpB* were reported previously (Soltis et al., 2000), as were several of the 26S rDNA sequences (see <http://ajbsupp.botany.org/v90/>); Kuzoff et al., 1998; Zanis et al., 2002). Most of the 26S rDNA sequences used here were generated as part of the present study. All DNA samples used were those employed in prior studies (e.g., Savolainen et al., 2000a; Soltis et al., 2000).

The entire 26S rDNA was amplified using the polymerase chain reaction (PCR) using primers N-nc26S1 and 3331rev (Kuzoff et al., 1998). In some instances, complete 26S rDNA sequences were obtained by amplifying the gene in two fragments with primer pairs N-nc26S1/1839rev and N-nc26S7/3331rev (Kuzoff et al., 1998). Amplified DNAs were purified with PEG/NaCl (Dunn and Blattner, 1987; Soltis et al., 1997) and used as templates in cycle sequencing with ABI PRISM BigDye (PE Biosystems, Foster City, California, USA). A subset of the primers used by Kuzoff et al. (1998) was sufficient to obtain complete 26S rDNA sequences: N-nc26S1, N-nc26S3, N-nc26S5, N-nc26S7, N-nc26S9, N-nc26S11, N-nc26S13, N-nc26S14, 268rev, 641rev, 950rev, 1499rev, 1839rev, 2426rev, 2782rev, 3331rev. Both DNA strands were sequenced. Sequencing was conducted on an ABI 377 automated sequencer (Applied Biosystems, Foster City, California, USA). Sequences were assembled and preliminary alignments constructed using Sequencher vers. 3.0 (Gene Codes, Ann Arbor, Michigan, USA).

As reported previously (Soltis et al., 2000), alignments of *rbcl*, *atpB*, and most of the 18S rDNA sequences were unambiguous. For *rbcl* and *atpB*, no gaps were required to align the sequences; for 18S rDNA, only four small regions corresponding to approximately 40 base pairs (bp) were difficult to align and were excluded from the analysis (Soltis et al., 2000). The 26S rDNA sequences also were aligned easily, except for a few short regions (expansion segments; discussed in Kuzoff et al., 1998); 155 of 3579 aligned 26S rDNA nucleotides were eliminated from the analysis. Of these omitted characters, 45 and 17 bp represent the 5' and 3' ends of the gene, respectively, regions corresponding in part to primer regions and for which many sequences are incomplete. The total length of the aligned four-gene data set was 8430 bp.

Phylogenetic analysis—Optimal topologies were obtained using maximum parsimony, as implemented in PAUP* 4.0 (Swofford, 1998). Several previous analyses have established that topologies based on the four individual genes (26S rDNA, 18S rDNA, *atpB*, *rbcl*) are largely congruent at deep levels in the angiosperms (e.g., Zanis et al., 2002), as well as within individual clades, including Saxifragales (Fishbein et al., 2001) and Ranunculales (S. Kim et al., unpublished manuscript). Hence, the four gene sequences were combined and analyzed phylogenetically. To ascertain the impact of the addition of 26S rDNA sequences, we removed the 26S rDNA sequences and analyzed the remaining three-gene data set.

Shortest trees were obtained using the heuristic search method and 1000 replicates of random taxon addition with tree bisection-reconnection (TBR) branching swapping, saving all shortest trees per replicate. Because of the large size of the data set, relative support for clades present was assessed using parsimony jackknifing (Farris et al., 1996). The version of the *Jac* program employed (Källersjö et al., 1998) has a faster tree-building algorithm than the original *Jac* program and also performs branch swapping. In this analysis, only clades with support greater than 50% were saved.

Data sets of this size are too large to analyze with either maximum likelihood or with Bayesian inference (Huelsenbeck et al., 2001). The program Mr. Bayes has enormous memory requirements; analyses employing Mr. Bayes depend on both the number of taxa and the number of characters. A data set of four genes (8430 characters) and 206 taxa is well beyond the current capabilities of the program. To run successfully a Bayesian analysis on a eudicot data set of four genes, we had to trim the number of taxa to 50, which then raises questions of adequate taxon density. Bayesian analyses were conducted using Mr. Bayes version 1.10 (Huelsenbeck, 2000). We used uniform prior probabilities and the general time-reversible + Γ model of molecular evolution. We ran four chains of the Markov Chain Monte Carlo, sampling every 100 generations for 40000 generations, starting with a random tree (more details can be obtained from the authors on request).

Character-state reconstruction—We examined perianth merosity to test the hypothesized lability of floral features in early-diverging eudicots using parsimony and MacClade version 3.04 (Maddison and Maddison, 1992). As a framework for character reconstruction, we used one of the shortest trees obtained in this analysis. We simplified the tree by reducing the number of terminals. This reduction is particularly evident for the core eudicot lineages, which we reduced to a single “summary” placeholder for the large subclades rosids, asterids, Caryophyllales, and Saxifragales. It is beyond the scope of this study to examine floral diversification in these large clades in any detail. The treatment of these clades as basically pentamerous in perianth merosity is consistent with the literature (e.g., Cronquist, 1981; Takhtajan, 1987), as well as with several recent reconstructions of floral characters (Ronse De Craene and Smets, 1993, 1995, 1998; M. Fishbein et al., unpublished manuscript). Other investigators have also followed this same approach in their consideration of the floral merosity of these large clades (Albert et al., 1998; Zanis et al., in press). We also reduced the number of early-diverging eudicots in the tree used for character state mapping. For example, we collapsed *Buxus* and *Pachysandra*, which are both mostly dimerous, to “Buxaceae” in our MacClade reconstruction.

We also examined the impact of alternative topologies on character-state reconstructions of perianth merosity. We considered alternative relationships (1) between Sabiaceae and Proteales (either as sister taxa or as successive sisters to remaining eudicots), (2) for Trochodendraceae (including *Tetracetrion*) and Buxaceae, treating them as a clade, or as successive sisters to the core eudicots, and (3) among Papaveraceae, Eupteleaceae, and the remaining Ranunculales. Although in this paper we illustrate Eupteleaceae followed by Papaveraceae as subsequent sisters to other Ranunculales (a result obtained herein; see also S. Kim et al., unpublished manuscript), we also explored the alternative of Papaveraceae followed by Eupteleaceae as successive sisters to other Ranunculales. Ultimately, these alternative reconstructions had no impact on the conclusions reached regarding the evolution of merosity in core eudicots.

Major sources for floral data are Drinnan et al. (1994), Endress (1986), Magallón-Puebla et al. (1997), and L. P. Ronse De Craene et al. (unpublished manuscript), and the primary references therein. Perianth merosity was scored as having the following unordered states: indeterminate, dimerous, trimerous, tetramerous, pentamerous, absent, or uncertain. We depict reconstructions using the “all most parsimonious states” trace option in MacClade, but we also used ACCTRAN optimization in which evolutionary changes are “accelerated” or permitted to occur early in evolutionary history and DELTRAN optimizations in which evolutionary changes are “delayed.”

RESULTS AND DISCUSSION

Phylogenetic relationships—Parsimony analyses of the four-gene data set resulted in 48 shortest trees, all in one island (Maddison, 1991), of 29 229 steps, consistency index (CI) = 0.200, retention index (RI) = 0.477 (Fig. 2). Parsimony analysis of the three gene data set (tree not shown) for the same set of taxa resulted in 12 shortest trees of length 29 059, CI = 0.201, RI = 0.479. Parsimony jackknife analyses were completed in only 3.03 h; the values obtained for the four-gene data set are provided (Fig. 2). A comparison of jackknife values obtained for the larger clades based on analyses of the four- and three-gene data sets is provided in Table 1; we do not provide a comparison of values within families or among most closely related families.

The four-gene topology for eudicots is very similar to that obtained here with three genes, as well as to that obtained in earlier analyses of two- and three-gene data sets (Hoot et al., 1999; Savolainen et al., 2000a; Soltis et al., 2000). We will focus mainly on those nodes obtaining jackknife support $\geq 50\%$. Jackknife support typically increased with the addition of 26S rDNA sequences, although in some instances support

remained unchanged or decreased with the addition of 26S rDNA sequences (Table 1).

The monophyly of the eudicots, as well as of many major clades (e.g., Saxifragales, Santalales, Caryophyllales, asterids, Ranunculales), is very strongly supported (99% or 100%) in the four-gene analysis, comparable to the results of other recent multi-gene analyses (Hoot et al., 1999; Soltis et al., 1999, 2000; Savolainen et al., 2000a). In the strict consensus of the shortest trees, the early-diverging eudicots form a grade with a strongly supported Ranunculales (100%) sister to all other eudicots. The addition of 26S rDNA sequences provides support (78%) for Eupteleaceae as sister to all other Ranunculales. In contrast, our analyses of three genes, as well as earlier analyses, placed Papaveraceae as sister to other Ranunculales, with comparable support (75%, Table 1; see also Hoot et al., 1999; Soltis et al., 2000).

Perhaps the most noteworthy result of the present analysis is the support for the placement of Gunnerales as the sister group to all remaining core eudicots, with jackknife support of 84%. In the three-gene analysis conducted here, support for this node was only 58%. Bayesian inference yielded a topology (not shown) essentially identical to that achieved with parsimony with Gunnerales sister to other core eudicots with a posterior probability of 0.99.

Previous analyses of one, two, or three genes revealed a similar placement of Gunnerales, but never with support $>50\%$. For example, one of the two analyses of Chase et al. (1993) placed Gunneraceae as sister to core eudicots (Myrothamnaceae were not included). Similarly, analyses of *rbcL* (Savolainen et al., 2000a) placed Gunneraceae + Myrothamnaceae as sister to all other core eudicots. However, other analyses of *rbcL* sequences placed Gunneraceae or Gunneraceae + Myrothamnaceae in different phylogenetic positions (see Chase et al., 1993; Savolainen et al., 2000b). For example, analysis of 18S rDNA sequences placed Gunneraceae (Myrothamnaceae were not included) + Dilleniaceae as sister to other core eudicots (Soltis et al., 1997). Analysis of *atpB* sequences (Savolainen et al., 2000a) placed a clade of (Gunneraceae + Myrothamnaceae) + Dilleniaceae as sister to all other core eudicots. An analysis of a combined *atpB* + *rbcL* data set also placed Gunneraceae + Myrothamnaceae as sister to all other core eudicots. Support for the monophyly of Gunnerales (Gunneraceae + Myrothamnaceae) also is higher in the four-gene analysis (85%) than in the three-gene analysis (77%) that we conducted (Table 1).

The addition of 26S rDNA sequences also increased support in other portions of the eudicot topology. The sister-group relationship of *Dillenia* + Caryophyllales increased from 72% with three genes to 83% with four genes (Table 1). Support also increases within some clades, such as within Saxifragales and Caryophyllales. In the former, there is very strong support (99%) for a clade of Crassulaceae, Tetracarpaceae, Penthoraceae, and Haloragaceae (see also Fishbein et al., 2001), which received lower (85%) support in the three-gene analysis. In Caryophyllales, support for the sister-group of *Triphyophyllum* + *Nepenthes* increased from 58% with three genes to 84% with four genes. There is also increased support with four genes for a nitrogen-fixing clade and a sister-group relationship between Malvales and Sapindales (Table 1). In our four-gene analysis, Cornales are the sister to all other asterids with 74% support, whereas our three-gene analysis, as well as most earlier analyses, did not find support $>50\%$ for this placement of Cornales (Savolainen et al., 2000a; Soltis et al., 2000).

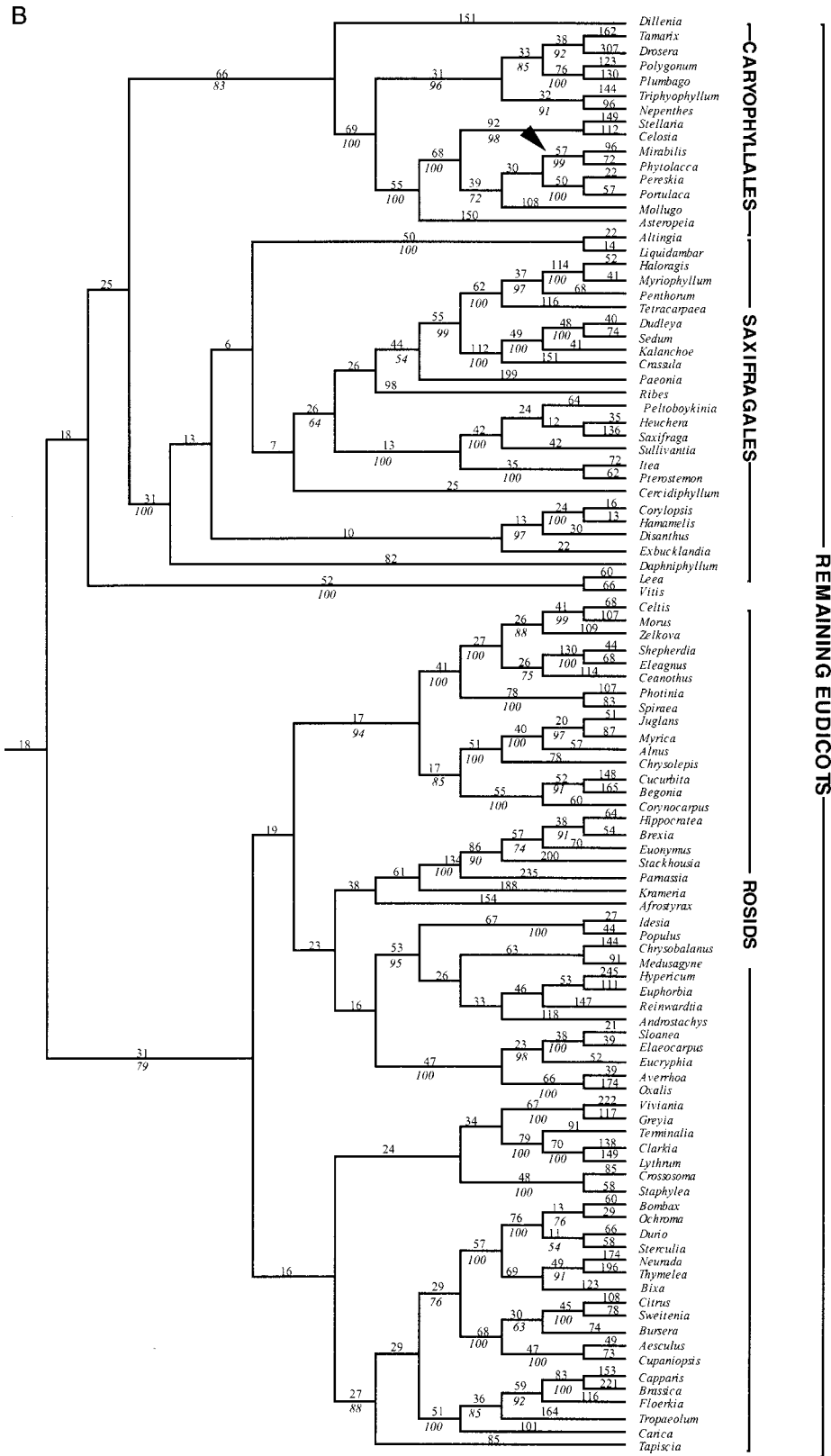


Fig. 2. Continued.

In other instances, internal support decreased with the addition of 26S rDNA. With four genes, *Leea* and *Vitis* (Vitaceae) appeared in the strict consensus as sister to the rest of the rosoid clade (the core rosoids in Table 1), but support for this placement is lower in the four-gene analysis (<50%) than in our three-gene analysis (58%; Table 1). Support for the monophyly of all rosoids except Vitaceae is also lower with four compared to three genes (97% vs. 84%), as is support for the monophyly of rosoid II (95% vs. 82%). Support for Proteales (73%) is lower with four genes than in our analysis of the same taxa with three genes (87%; see also Savolainen et al., 2000a; Soltis et al., 2000). Our analysis of 26S rDNA sequences alone placed *Nelumbo* with Proteaceae, rather than placing Platanaceae with Proteaceae, as was found in earlier analyses (e.g., Soltis et al., 2000; Savolainen et al., 2000a, b). There appears, therefore, to be some conflict in signal with the addition of 26S rDNA.

The reasons for these occasional instances of decreased support for relationships with the addition of 26S rDNA remain unclear. Similar examples of occasional decreased support (or no change in support) were observed in the combination of 18S rDNA, *atpB*, and *rbcL* data sets (Soltis et al., 1998). This decrease could be an artifact of taxon sampling. Some subclades of rosoids, for example, are poorly represented, or not represented at all, in the current analysis. For example, Fabales are not represented in the present analysis. Another factor could be unequal rates of base substitution between expansion segments and conserved core regions (Kuzoff et al., 1998). Although we eliminated parts of the expansion segments that were difficult to align, we did use some easy-to-align portions of the 26S rDNA expansion segments. Unequal rates of base substitution between the expansion segments and conserved core regions could affect phylogeny reconstruction in parsimony analyses (e.g., Wakeley, 1996; Yang, 1996). Although it might be possible to compensate for these unequal rates with maximum-likelihood estimation (e.g., Yang, 1996; Lewis, 1998), such analyses are not feasible with data sets of this size. Additional sequences of 26S rDNA, as well as more detailed assessments of the molecular evolution of 26S rDNA sequences, may help to clarify the relationships that received lower support with the addition of 26S rDNA sequences.

Although our results provide the first support for the placement of Gunnerales as sister to all other core eudicots, no other relationships among major core eudicot lineages received substantive support with the four-gene data set. There is very weak support (58%) for a placement of Berberidopsidales as sister to all other core eudicots following Gunnerales. Furthermore, with four genes, Saxifragales appear in the strict consensus (but without support $\geq 50\%$) as sister to all other eudicots following Berberidopsidales. In contrast, with three genes, Saxifragales appeared with weak support (60%) as sister to the rosoids (Soltis et al., 2000). Thus, relationships among the remaining clades of core eudicots (Berberidopsidales, Caryophyllales, Saxifragales, Santalales, asterids, rosids) remain unclear. Resolving relationships among these remaining major lineages of core eudicots, which comprise the vast majority of all extant angiosperms, should be a major priority of angiosperm phylogenetic analyses. Not only is an understanding of core eudicot relationships critical to angiosperm systematics, but it is also essential for an improved understanding of character evolution across the eudicots. The fact that core eudicot clades essentially form a polytomy compromises efforts of large-scale character-state reconstruction. Resolving relation-

ships among these eudicot clades will be a major undertaking, requiring the use of several additional genes that will need to be sequenced for hundreds of taxa. For comparison, critical relationships among many of the major lineages of basal angiosperms were ultimately resolved with strong support using an 11-gene data set (Zanis et al., 2002). However, the basal angiosperms consist of a much smaller number of taxa than do the core eudicots. Furthermore, a starburst topology may accurately depict relationships among major lineages of core eudicots. Core eudicots may, in fact, represent an ancient, rapid radiation, and even with the addition of more gene sequences, relationships will not be resolved. Analyses of sequence data suggest that this is the case in other instances, such as for deep-level relationships in Saxifragales (Fishbein et al., 2001).

Evolution of perianth merosity—The position of Gunnerales as sister to all other eudicots has important implications for floral evolution. A common view of floral evolution is that some features, most notably merosity (merism), is highly labile in early-diverging eudicots and becomes canalized in the core eudicots, a clade in which most taxa are five-merous (Endress, 1987a, b, 1990; Drinnan et al., 1994; Ronse De Craene and Smets, 1994; Albert et al., 1998). Indeed, there is ample evidence to suggest that pentamery constitutes the basic merosity in the major lineages of core eudicots (e.g., Caryophyllales, Santalales, Saxifragales, asterids, rosids). For example, character state reconstructions using parsimony indicate that pentamery is the ancestral merosity for the core eudicot clade Saxifragales (M. Fishbein et al., unpublished manuscript). In core eudicots there is sometimes variation between a pentamerous and tetramerous perianth merosity, but there is not a dimerous perianth (in contrast to the early-branching eudicots). Taxa with trimerous flowers occur occasionally in several families of core eudicots, but these are nested within groups characterized by tetramery and pentamery (Endress, 1996). There are many highlighted examples of transitions of pentamery to tetramery (or other merosities) and the mechanisms for the transitions are well understood (e.g., Ronse De Craene and Smets, 1994; Endress, 1999). Most commonly, merosities lower than five arise by a fusion of parts (e.g., *Mollugo*: Batenburg and Moeliono, 1982; *Tripetaleia*: Nishino, 1988), or by loss of a sector of the flower (e.g., *Geum*: Ronse De Craene and Smets, 1994), or a combination of fusion and loss (Capparaceae: Ronse De Craene, 2002), while higher merosities are the result of a lateral increase affecting a series of whorls in the flower.

An in-depth discussion of general patterns of floral diversification in early-diverging eudicots was provided by Drinnan et al. (1994), who provided several hypotheses of floral evolution, although without explicit phylogenetic reconstructions of character evolution. Our reconstructions confirm the suggestions of earlier investigators (Endress, 1987a, b, 1994; Drinnan et al., 1994) of a high lability in floral form and merosity in early-diverging eudicots (Fig. 3). This finding for early-diverging eudicots is similar to the lability in merosity reconstructed for basal angiosperms (Albert et al., 1998; Zanis et al., in press; L. P. Ronse De Craene et al., unpublished manuscript). A dimerous perianth is common within the early-diverging eudicots and may, in fact, be ancestral for these plants, although this reconstruction depends in part on the coding of Proteaceae and the optimization method used. Importantly, however, these factors have no impact on the recon-

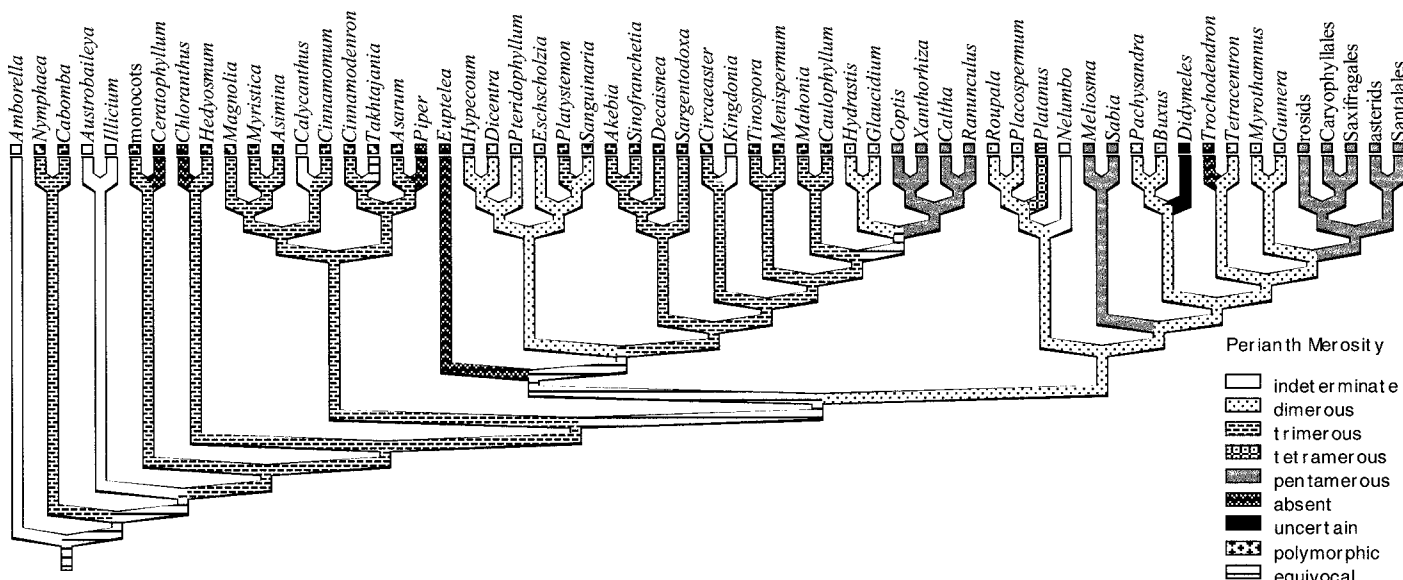


Fig. 3. MacClade reconstruction of the evolution of perianth merosity.

struction of the evolution of merosity in core eudicots and their immediate ancestors.

The perianth in Proteaceae has sometimes been interpreted as tetramerous (Drinnan et al., 1994), but phyllotactically, the four tepals “could represent two dimerous whorls of successive primordia” (Douglas and Tucker, 1996). The pattern of perianth phyllotaxis in Proteaceae is similar to that observed in Buxaceae and Papaveraceae, which have dimerous (opposite/decussate) arrangements of primordia (Douglas and Tucker, 1996). Thus, the best interpretation of merism in Proteaceae appears to be dimery (see also L. P. Ronse De Craene et al., unpublished manuscript). If Proteaceae are tetramerous, they represent one of the few instances in which this arrangement has evolved in early-diverging eudicots. Some Ranunculaceae appear to be tetramerous (Drinnan et al., 1994), although they have a similar dimerous arrangement of primordia (L. P. Ronse De Craene, personal observation). Ranunculaceae are highly variably in merosity, and trimerous, pentamerous, or dimerous flowers are abundant and occur in different genera (e.g., *Eranthis*, *Cimicifuga*, *Clematis*; Salisbury, 1919; Schöffel, 1932; Endress, 1987b; Ronse De Craene and Smets, 1993). Platanaceae, the sister group to Proteaceae in most molecular analyses (e.g., Chase et al., 1993; Savolainen et al., 2000a, b; Soltis et al., 2000), may be tetramerous; early and late Cretaceous fossils of Platanaceae are tetramerous and pentamerous (Friis et al., 1988; Crane et al., 1993; Magallón-Puebla et al., 1997). It is noteworthy that a staminate inflorescence of platanaceous affinity found associated with *Tanyoplatanus* infructescences differs from other Cretaceous Platanaceae in having two stamens per flower (Friis and Endress, 1996). Modern Platanaceae are tetramerous, trimerous, or irregular (e.g., Boothroyd, 1930). *Tetracentron* possesses a dimerous perianth, whereas a perianth appears to be absent from its sister group, *Trochodendron*. However, rudiments of what may be tepals are present in variable number and position in very young flowers of *Trochodendron* (Endress, 1986). A dimerous perianth may also be ancestral for Buxaceae (which includes *Didymeles*; see APG II, 2002). Most staminate Buxaceae are dimerous with two stamen whorls, although pistillate flowers are either trim-

erous or dimerous (van Tieghem, 1897). The weakly differentiated perianth is spiral in pistillate flowers, such as *Pachysandra* or *Sarcococca*, while two whorls are present in staminate flowers. Staminate *Styloceras* are unusual in lacking a perianth and having up to more than 30 stamens per flower (von Balthazar and Endress, 2002). *Didymeles* possesses one to four scale-like perianth parts in pistillate flowers; staminate flowers lack a perianth. We have scored the merosity of *Didymeles* as uncertain (Fig. 3). Buxaceae and Trochodendraceae form either a clade or a grade and are the immediate sister families to the large clade of Gunnerales + other core eudicots.

Gunnerales are noteworthy in that they share a dimerous perianth merosity with many early-diverging eudicots. Contrary to most other basal eudicots, Gunneraceae have a calyx and corolla with the stamens opposite the corolla. As such, the flower has been interpreted as obhaplostemonous, especially in the context of a presumed rosid relationship (Ronse De Craene and Smets, 1995). However, this condition appears to be highly unusual and dependent on the definition of the calyx and corolla in the family. Sepals may represent bracteoles forming a cupule around the inferior gynoecium. This possibility is substantiated by the transversal position of the styles, giving a distichous arrangement of petals, stamens, and carpels. In the related Myrothamnaceae, a perianth may be absent or is only loosely integrated in the flower, and the merosity has been variously interpreted as dimerous or labile (Endress, 1989; Drinnan et al., 1994; Jäger-Zürn, 1966). Jäger-Zürn (1966) interpreted the scales around stamens or carpels in *Myrothamnus* as bracts; thus, the flowers would be considered to lack a perianth. Male flowers have four stamens in *M. moschata*, three to eight stamens in *M. flabellifolia*. Female flowers have mostly four carpels, although sometimes there are three carpels (e.g., carpels are mostly three in *M. moschata*). Thus, the number of floral organs appears to be labile in *Myrothamnus*. Drinnan et al. (1994) considered *Myrothamnus* to be dimerous. It could well be that the basic number in the genus is two if one takes into account that the stamens or carpels, respectively (if four are present), are superposed to

the preceding bracts and do not alternate with them. Because of the uncertainty surrounding the merosity of *Myrothamnus*, we experimented with two different codings in our character-state reconstructions, dimerous and uncertain.

We considered Proteaceae to be dimerous (Fig. 3), but we also considered the impact of the alternative coding of Proteaceae as tetramerous. With Proteaceae scored as dimerous, ACCTRAN optimizations indicate that the dimerous perianth is ancestral for all eudicots; it is also reconstructed as ancestral for the node to Proteaceae, Sabiaceae, Buxaceae, Trochodendraceae, Gunnerales, and all core eudicots. With other approaches to tracing character evolution such as DELTRAN or all most-parsimonious states at nodes, dimery is not reconstructed as ancestral for the entire eudicot clade. Using the all most-parsimonious states at nodes optimization option, the ancestral state for the eudicots is ambiguous; however, the branch to Proteaceae, Sabiaceae, Buxaceae, Trochodendraceae, Gunnerales, and all core eudicots is reconstructed as dimerous (Fig. 3).

As noted, Proteaceae may be best scored as dimerous, and the alternative scoring of Proteaceae as tetramerous has an impact on character-state reconstruction (not shown). The ACCTRAN optimization results are similar to those noted earlier. A dimerous perianth is ancestral for all eudicots; it is also reconstructed as ancestral for Sabiaceae, Buxaceae, Trochodendraceae, Gunnerales, and all core eudicots, but the ancestral condition for Proteales is ambiguous. Using the all most-parsimonious states at nodes optimization option, the ancestral state for the eudicots is ambiguous, as is the common ancestor of Proteales and all remaining eudicots; however, the branch to Buxaceae, Trochodendraceae, Gunnerales, and all other core eudicots is again reconstructed as dimerous. With DELTRAN optimization, the ancestral state of the eudicots is reconstructed as trimerous, which is also frequent in the early-diverging eudicots, as well as in basal angiosperms (Albert et al., 1998; Zanis et al., in press). However, the branch to Buxaceae, Trochodendraceae, Gunnerales, and all other core eudicots is reconstructed as dimerous.

Regardless of the coding of Proteaceae or *Myrothamnus*, or optimization method, the pentamerous perianth has arisen at least three times within the early-diverging eudicots (Fig. 3): once in Sabiaceae, once in the ancestor of the sister clade to Gunnerales, and at least twice within Ranunculaceae (S. Hoot, University of Wisconsin–Milwaukee, personal communication). A dimerous perianth may also be ancestral for Ranunculaceae (Drinnan et al., 1994); however, reconstruction of the ancestral state of the family is equivocal (Fig. 3). *Hydrastis* and *Glaucidium*, two dimerous genera, are sister to the remainder of the family, which in our analyses is a clade (*Coptis*, *Xanthorrhiza*, *Caltha*, and *Ranunculus*), consisting entirely of pentamerous taxa. More detailed studies of Ranunculaceae are needed to determine the number of times a pentamerous perianth has arisen in the family. As in the basal angiosperms (Albert et al., 1998; Zanis et al., in press), a trimerous perianth is also common in the early-diverging eudicots. Reconstructions using ACCTRAN optimization indicate that a trimerous perianth is the ancestral state for most members of Ranunculales other than Papaveraceae (Fig. 3) and, as noted, with Proteaceae scored as tetramerous, DELTRAN optimization reconstructs the ancestral state for all eudicots as trimerous.

Although a dimerous or trimerous perianth is frequently encountered in early-diverging eudicots (e.g., Ranunculales, Proteales, Trochodendraceae, Buxaceae), in most core eudicots the

pentamerous condition predominates. Importantly, a dimerous perianth is also found in Gunneraceae and perhaps Myrothamnaceae, and all reconstructions, regardless of the optimization, topology, or coding of Proteaceae or *Myrothamnus*, indicate that a dimerous perianth is the immediate precursor of the pentamerous condition characteristic of core eudicots (Fig. 3). This result is important in that it indicates that the canalization of merosity that yielded the pentamerous perianth typical of core eudicots occurred following the divergence of Gunnerales from the rest of the core eudicots.

It is important to know how pentamery has been derived from dimery in a structural way. Different possibilities have been presented by L. P. Ronse De Craene et al. (unpublished manuscript). In several families of early-diverging eudicots, dimerous flowers co-occur with trimerous flowers in closely related genera or species. In some instances one also finds pentamerous flowers (e.g., Ranunculaceae, Berberidaceae, Menispermaceae, Buxaceae). Pentamery could be seen as an intermediate condition between trimery and dimery by loss of a perianth member or union of two intermediate members (Ronse De Craene and Smets, 1994). The report of a fair number of pentamerous flower buds in the normally dimerous *Persoonia falcata* of Proteaceae by Douglas and Tucker (1996) indicates the possibility of an easy transition between dimery and pentamery. The transition between a decussate and spiral phyllotaxis is caused by an increase in size of the apex and the insertion of a primordium on the adaxial side of the flower. If a dimerous merosity is ancestral for the core eudicots, pentamery could be derived by the superposition of a dimerous and a trimerous whorl by a change of phyllotaxis caused by the addition of one organ (cf. Endress, 1987b; Drinnan et al., 1994).

Our reconstructions of floral evolution support the suggestion of Drinnan et al. (1994) that lability in floral form was prevalent in the early-diverging eudicots. They suggested that dimerous (opposite/decussate) and trimerous (ternate) arrangements are widespread in early eudicots and “likely primitive” and that the transition from one to the other may have occurred multiple times. Furthermore, the origin of pentamery in both the core eudicots and pentamerous Ranunculaceae appears to have involved dimerous ancestors (Fig. 3); the origin of pentamery in Sabiaceae is unclear.

LITERATURE CITED

- ALBACH, D. C., P. S. SOLTIS, D. E. SOLTIS, AND R. G. OLMSTEAD. 2001. Phylogenetic analysis of the Asteridae s. l. based on sequences of four genes. *Annals of the Missouri Botanical Garden* 88: 163–212.
- ALBERT, V. A., M. H. G. GUSTAFFSON, AND L. DILAURENZIO. 1998. Ontogenetic systematics, molecular developmental genetics, and the angiosperm petal. In D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], *Molecular systematics of plants II*, 349–374. Kluwer Academic, New York, New York, USA.
- APG. 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* 85: 531–553.
- APG II. 2002. An updated classification of the angiosperms. *Botanical Journal of the Linnean Society*, in press.
- BARKMAN, T. J., G. CHENERY, J. R. MCNEAL, J. LYONS-WEILER, W. J. ELLISENS, G. MOORE, A. D. WOLFE, AND C. W. DEPAMPHILIS. 2000. Independent and combined analyses of sequences from all three genomic compartments converge on the root of flowering plant phylogeny. *Proceedings of the National Academy of Sciences, USA* 97: 13166–13171.
- BATENBURG, L. H., AND B. M. MOELIENO. 1982. Oligomery and vasculature in the androecium of *Mollugo nudicaulis* Lam. (Molluginaceae). *Acta Botanica Neerlandica* 31: 215–220.

- BOOTHROYD, L. E. 1930. The morphology and anatomy of the inflorescence and flower of the Platanaceae. *American Journal of Botany* 17: 678–693.
- BOWE, L. M., G. COAT, AND C. W. DEPAMPHILIS. 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 of Sciences, USA* 97: 4092–4097.
- CHASE, M. W., AND V. A. ALBERT. 1998. A perspective on the contribution of plastid *rbcL* DNA sequences to angiosperm phylogenetics. In D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], *Molecular systematics of plants II*, 489–507. Kluwer Academic, New York, New York, USA.
- CHASE, M. W., D. E. SOLTIS, P. S. SOLTIS, P. J. RUDALL, M. F. FAY, W. H. HAHN, S. SULLIVAN, J. JOSEPH, T. GIVNISH, K. J. SYTSMA, AND C. PIRES. 2000. Higher-level systematics of the monocotyledons: an assessment of current knowledge and a new classification. In K. Wilson and D. Morrison [eds.], *Proceedings of monocots II: the second international symposium on the comparative biology of the monocotyledons*, 3–16. CSIRO Press, Sydney, Australia.
- CHASE, M. W., ET AL. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 528–580.
- CRANE, P. R., K. R. PEDERSEN, E. M. FRIIS, AND A. N. DRINNAN. 1993. Early Cretaceous (early to middle Albian) platanoid inflorescences associated with *Sapindopsis* leaves from the Potomac Group of eastern North America. *Systematic Botany* 18: 328–344.
- CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, New York, USA.
- DOUGLAS, A., AND S. C. TUCKER. 1996. Comparative floral ontogenies among Persoonioideae including *Bellendena* (Proteaceae). *American Journal of Botany* 83: 1528–1555.
- DRINNAN, A. N., P. R. CRANE, AND S. B. HOOT. 1994. Patterns of floral evolution in the early diversification of non-magnoliid dicotyledons (eudicots). *Plant Systematics and Evolution*, Supplement 8: 93–122.
- DUNN, I. S., AND F. R. BLATTNER. 1987. Charons 36 to 40: multi enzyme, high capacity, recombination deficient replacement vectors with poly-linkers and polystuffers. *Nucleic Acids Research* 15: 2677–2698.
- ENDRESS, P. K. 1986. Reproductive structures and phylogenetic significance of extant primitive angiosperms. *Plant Systematics and Evolution* 152: 1–28.
- ENDRESS, P. K. 1987a. The early evolution of the angiosperm flower. *Trends in Ecology and Evolution* 2: 300–304.
- ENDRESS, P. K. 1987b. Floral phyllotaxis and floral evolution. *Botanische Jahrbücher für Systematik* 108: 417–438.
- ENDRESS, P. K. 1989. The systematic position of the Myrothamnaceae. In P. R. Crane and S. Blackmore [eds.], *Evolution, systematics, and fossil history of the Hamamelidae*, vol. 1: introduction and 'lower' Hamamelidae. Systematics Association Special volume No 40A, 193–200. Clarendon Press, Oxford, UK.
- ENDRESS, P. K. 1990. Patterns of floral construction in ontogeny and phylogeny. *Biological Journal of the Linnean Society* 39: 153–175.
- ENDRESS, P. K. 1994. Floral structure and evolution of primitive angiosperms: recent advances. *Plant Systematics and Evolution* 192: 79–97.
- ENDRESS, P. K. 1996. Homoplasy in angiosperm flowers. In M. J. Sanderson and L. Hufford [eds.], *Homoplasy: the recurrence of similarity in evolution*, 303–325. Academic Press, San Diego, California, USA.
- ENDRESS, P. K. 1999. Symmetry in flowers: diversity and evolution. *International Journal of Plant Science* 160(Supplement): S3–S23.
- ERBAR, C., AND P. LEINS. 1981. Zur spirale in Magnolienblüten. *Beiträge zur Biologie der Pflanzen* 56: 225–241.
- ERBAR, C., AND P. LEINS. 1994. Flowers in Magnoliidae and the origin of flowers in other subclasses of the angiosperms. I. The relationships between flowers of Magnoliidae and Alismatidae. *Plant Systematics and Evolution* (Supplement) 8: 193–208.
- FARRIS, J. S., V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.
- FISHBEIN, M., C. HIBSCH-JETTER, D. E. SOLTIS, AND L. HUFFORD. 2001. Phylogeny of Saxifragales (angiosperms, eudicots): analysis of a rapid, ancient radiation. *Systematic Biology* 50: 817–847.
- FRIIS, E. M., P. R. CRANE, AND K. R. PEDERSEN. 1988. Reproductive structure of Cretaceous Platanaceae. *Biologiske Skrifter kongelige Danske Videnskaberne Selskab* 31: 1–55.
- FRIIS, E. M., AND P. K. ENDRESS. 1996. Flower evolution. In H.-D. Behnke, U. Lüttge, K. Esser, J. W. Kadereit, and M. Runge [eds.], *Progress in botany* 57, 253–280. Springer-Verlag, Berlin, Germany.
- GRAHAM, S. W., AND R. G. OLMSTEAD. 2000. Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *American Journal of Botany* 87: 1712–1730.
- HOOT, S. B., S. MAGALLON, AND P. R. CRANE. 1999. Phylogeny of basal eudicots based on three molecular datasets: *atpB*, *rbcL*, and 18S nuclear ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* 86: 1–32.
- HUELSENBECK, J. P. 2000. Mr. Bayes. Distributed by the author. Department of Biology, University of Rochester, Rochester, New York, USA.
- HUELSENBECK, J. P., F. RONQUIST, R. NIELSEN, AND J. P. BOLLBACK. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310–2314.
- JÄGER-ZÜRN, I. 1966. Infloreszenz- und blütenmorphologische, sowie embryologische Untersuchungen an *Myrothamnus* Welw. *Beiträge zur Biologie der Pflanzen* 42: 241–271.
- KÄLLERSJÖ, M., J. S. FARRIS, M. W. CHASE, B. BREMER, M. F. FAY, C. J. HUMPHRIES, G. PETERSEN, O. SEBERG, AND K. BREMER. 1998. Simultaneous parsimony jackknife analysis of 2538 *rbcL* DNA sequences reveals support for major clades of green plants, land plants, seed plants, and flowering plants. *Plant Systematics and Evolution* 213: 259–287.
- KUBITZKI, K., 1987. Origin and significance of trimerous flowers. *Taxon* 36: 21–28.
- KUZOFF, R. K., J. A. SWEERE, D. E. SOLTIS, P. S. SOLTIS, AND E. A. ZIMMER. 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. *Molecular Biology and Evolution* 15: 251–263.
- LEWIS, P. O. 1998. Maximum likelihood as an alternative to parsimony for inferring phylogeny using nucleotide sequence data. In D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], *Molecular systematics of plants II*, 132–163. Kluwer Academic, New York, New York, USA.
- MADDISON, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology* 40: 315–328.
- MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.
- MAGALLÓN-PUEBLA, S., P. S. HERENDEEN, AND P. R. CRANE. 1997. *Quadriplatanus georgianus* gen. et sp. nov.: staminate and pistillate platanaceous flowers from the Late Cretaceous (Coniacian-Santonian) of Georgia, U.S.A. *International Journal of Plant Sciences* 158: 373–394.
- MATHEWS, S., AND M. J. DONOGHUE. 1999. The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286: 947–949.
- NEYLAND, R. 2001. A phylogeny inferred from large ribosomal subunit (26S) rDNA sequences suggests that *Cuscuta* is a derived member of Convolvulaceae. *Brittonia* 53: 108–115.
- NICKRENT, D. L., AND V. MALÉCOT. 2001. A molecular phylogeny of Santalales. In A. Fer, P. Thalouarn, D. M. Joel, L. J. Musselman, C. Parker, and J. A. C. Verkleij [eds.], *Proceedings of the 7th International Parasitic Weed Symposium*, 69–74. Faculté des Sciences, Université de Nantes, Nantes, France.
- NISHINO, E. 1988. Early floral organogenesis in *Tripetaleia* (Ericaceae). In P. Leins, S. C. Tucker, and P. K. Endress [eds.], *Aspects of floral development*, 181–190. Cramer, Berlin, Germany.
- OLMSTEAD, R. G., R. K. JANSEN, K. J. KIM, AND S. J. WAGSTAFF. 2000. The phylogeny of the Asteridae s. l. based on chloroplast *ndhF* sequences. *Molecular Phylogenetics and Evolution* 16: 96–112.
- PARKINSON, C. L., K. L. ADAMS, AND J. D. PALMER. 1999. Multigene analyses identify the three earliest lineages of extant flowering plants. *Current Biology* 9: 1485–1488.
- QIU, Y.-L., M. W. CHASE, S. B. HOOT, E. CONTI, P. R. CRANE, K. J. SYTSMA, AND C. R. PARKS. 1998. Phylogenetics of the Hamamelidae and their allies: parsimony analyses of nucleotide sequences of the plastid gene *rbcL*. *International Journal of Plant Science* 159: 891–905.
- QIU, Y.-L., J. LEE, F. BERNASCONI-QUADRONI, D. E. SOLTIS, P. S. SOLTIS, M. ZANIS, E. A. ZIMMER, Z. CHEN, V. SAVOLAINEN, AND M. W. CHASE. 1999. The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* 402: 404–407.
- QIU, Y.-L., J. LEE, F. BERNASCONI-QUADRONI, D. E. SOLTIS, P. S. SOLTIS, M. ZANIS, E. A. ZIMMER, Z. CHEN, V. SAVOLAINEN, AND M. W. CHASE. 2000. Phylogenetic analyses of basal angiosperms based on five genes from all three genomes. *International Journal of Plant Sciences* 161: S3–S27.
- RONSE DE CRAENE, L. P. 2002. Floral development and anatomy of *Penta-*

- diplandra* (Pentadiplandraceae): a key genus in the identification of floral morphological trends in the core Brassicales. *Canadian Journal of Botany* 80: in press.
- RONSE DE CRAENE, L. P., AND E. SMETS. 1993. The distribution and systematic relevance of the androecial character polymery. *Botanical Journal of the Linnean Society* 113: 285–350.
- RONSE DE CRAENE, L. P., AND E. SMETS. 1994. Merosity in flowers: definition, origin and taxonomic significance. *Plant Systematics and Evolution* 191: 83–104.
- RONSE DE CRAENE, L. P., AND E. SMETS. 1995. Evolution of the androecium in the Ranunculiflorae. *Plant Systematics and Evolution* (Supplement) 9: 63–70.
- RONSE DE CRAENE, L. P., AND E. SMETS. 1998. Meristic changes in gynoecium morphology, exemplified by floral ontogeny and anatomy. In S. J. Owens and P. J. Rudall [eds.], *Reproductive biology*, 85–112. Royal Botanic Gardens, Kew, UK.
- SALISBURY, E. J. 1919. Variation in *Eranthis hyemalis*, *Ficaria verna*, and other members of the Ranunculaceae, with special reference to trimery and the origin of the perianth. *Annals of Botany* 33: 47–79.
- SAVOLAINEN, V., M. W. CHASE, C. M. MORTON, S. B. HOOT, D. E. SOLTIS, C. BAYER, M. F. FAY, A. DE BRUIJN, S. SULLIVAN, AND Y.-L. QIU. 2000a. Phylogenetics of flowering plants based upon a combined analysis of plastid *atpB* and *rbcL* gene sequences. *Systematic Biology* 49: 306–362.
- SAVOLAINEN, V., ET AL. 2000b. Phylogeny of the eudicots: a nearly complete familial analysis based on *rbcL* gene sequences. *Kew Bulletin* 55: 257–309.
- SCHÖFFEL, K. 1932. Untersuchungen über den Blütenbau der Ranunculaceen. *Planta* 17: 315–371.
- SOLTIS, D. E., P. S. SOLTIS, M. E. MORT, M. W. CHASE, V. SAVOLAINEN, S. B. HOOT, AND C. M. MORTON. 1998. Inferring complex phylogenies using parsimony: an empirical approach using three large DNA datasets for angiosperms. *Systematic Biology* 47: 32–42.
- SOLTIS, D. E., R. K. KUZOFF, M. E. MORT, M. ZANIS, M. FISHBEIN, L. HUFFORD, J. KOONTZ, AND M. K. ARROYO. 2001. Elucidating deep-level phylogenetic relationships in Saxifragaceae using sequences for six chloroplast and nuclear DNA regions. *Annals of the Missouri Botanical Garden* 88: 669–693.
- SOLTIS, D. E., ET AL. 1997. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* 84: 1–49.
- SOLTIS, D. E., ET AL. 2000. Angiosperm phylogeny inferred from a combined data set of 18S rDNA, *rbcL* and *atpB* sequences. *Botanical Journal of the Linnean Society* 133: 381–461.
- SOLTIS, P. S., D. E. SOLTIS, AND M. W. CHASE. 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402: 402–404.
- SWOFFORD, D. L. 1998. PAUP*: phylogenetic analysis using parsimony, version 4.0. Sinauer, Sunderland, Massachusetts, USA.
- TAKHTAJAN, A. 1987. System of Magnoliophyta. Academy of Sciences U.S.S.R., Leningrad, Russia.
- VAN TIEGHEM, P. 1897. Sur les Buxacées. *Annales des Sciences naturelles Botanique*. Série 8, 5: 289–338.
- VON BLATHAZAR, M., AND P. K. ENDRESS. 2002. Development of inflorescences and flowers in Buxaceae and the problem of perianth interpretation. *International Journal of Plant Sciences* 163: 847–876.
- WAKELEY, J. 1996. The excess of transitions among nucleotide substitutions: new methods of estimating transition bias underscore its significance. *Trends in Ecology and Evolution* 11: 158–163.
- YANG, Z. 1996. Among site variation and its impact on phylogenetic analysis. *Trends in Ecology and Evolution* 11: 367–372.
- ZANIS, M. J., D. E. SOLTIS, P. S. SOLTIS, S. MATHEWS, AND M. J. DONOGHUE. 2002. The root of the angiosperms revisited. *Proceedings of the National Academy of Sciences, USA* 99: 6848–6853.
- ZANIS, M. J., P. S. SOLTIS, Y.-L. QIU, E. ZIMMER, AND D. E. SOLTIS. In press. Phylogenetic analyses and perianth evolution in basal angiosperms. *Annals of the Missouri Botanical Garden*.