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PHYLOGENETIC STRUCTURE IN THE GRASS FAMILY (POACEAE) AS INFERRED FROM CHLOROPLAST DNA RESTRICTION SITE VARIATION¹

JERROLD I DAVIS² AND ROBERT J. SORENG

L. H. Bailey Hortorium, Cornell University, Ithaca, New York 14853

A cladistic analysis of chloroplast DNA restriction site variation among representatives of all subfamilies of the grass family (Poaceae), using *Joinvillea* (Joinvilleaceae) as the outgroup, placed most genera into two major clades. The first of these groups corresponds to a broadly circumscribed subfamily Pooideae that includes all sampled representatives of Ampelodesmeae, Aveneae, Brachypodioideae, Bromaeae, Diarrheneae, Meliceae, Poeae, Stipeae, and Triticeae. The second major clade includes all sampled representatives of four subfamilies (Panicoideae [tribes Andropogoneae and Paniceae], Arundinoideae [Arundineae], Chloridoideae [Eragrostideae], and Centothecoideae [Centothecoaceae]). Within this group (the "PACC" clade), the Panicoideae are resolved as monophyletic and as the sister group of the clade that comprises the other three subfamilies. Within the latter group, *Danthonia* (Arundinoideae) and *Eragrostis* (Chloridoideae) are resolved as a stable monophyletic group that excludes *Phragmites* (Arundinoideae); this structure is inconsistent with the Arundinoideae being monophyletic as currently circumscribed. The PACC clade is placed within a more inclusive though unstable clade that includes the woody Bambusoideae (Bambuseae) plus several disparate tribes of herbaceous grasses of uncertain affinity that are often recognized as herbaceous Bambusoideae (Brachyelytreae, Nardeae, Olyreae, Oryzae, and Phareae). Among eight most-parsimonious trees resolved by the analysis, four include a monophyletic Bambusoideae sensu lato (comprising Bambuseae and all five of these herbaceous tribes) as the sister group of the PACC clade; in the other four trees these bambusoid elements are not resolved as monophyletic, and the PACC clade is nested among these tribes. These results are consistent with those of previous analyses that resolve a basal or near-basal branch within the family between Pooideae and all other grasses. However, resolution by the present analysis of the PACC clade, which includes Centothecoideae, Chloridoideae, and Panicoideae, but excludes Bambusoideae, is inconsistent with the results of previous analyses that place Bambusoideae and Panicoideae in a monophyletic group that excludes Centothecoideae and Chloridoideae.

Since their origin, no later than the Eocene (Crepet and Feldman, 1991), and possibly as early as the Upper Cretaceous (Linder, 1987), the grasses (Poaceae) have come to occupy virtually every habitat that supports vascular plant life, often as overwhelming dominants. Approximately 10,000 grass species are currently recognized, apportioned among 650 to more than 700 genera (Clayton and Renvoize, 1986; Watson and Dallwitz, 1992). Syntheses of the enormous literature describing character variation among grasses have resulted in a detailed taxonomic structure that accommodates most of these genera within five to seven subfamilies, with the larger subfamilies further subdivided into numerous tribes and informal generic groupings (Stebbins, 1956, 1987; Tateoka, 1957a; Stebbins and Crampton, 1961; Clayton, 1981; Clayton and Renvoize, 1986; Watson and Dallwitz, 1992). Several additional genera of questionable affinity (e.g., *Brachyelytrum* [Campbell, Garwood, and Specht, 1986]) are variously placed in loose affiliation with one or another of

the conventional subfamilies or recognized as constituting additional minor subfamilies (e.g., Centothecoideae [Decker, 1964a; Soderstrom and Decker, 1973; Soderstrom, 1981b; Clayton and Renvoize, 1986]). Although differences among taxonomic schemes highlight specific areas of uncertainty concerning relationships, the proper placement of anomalous genera among the established subfamilies is only an element of a larger problem, which is to resolve the overall pattern of phylogenetic relationships within this diverse family and to establish a classification system compatible with this phylogeny. The principal subfamilies—or at least the major elements of them—are united by correlated sets of features, in many cases reasonably characterized as synapomorphic, but a phylogenetic structure that depicts relationships among the subfamilies and the anomalous genera has been elusive.

Conflicting phylogenetic hypotheses concerning the grasses are best addressed with reference to the principal groups that are conventionally recognized and to the various and conflicting combinations in which their constituent taxa have been grouped by various authorities. It should be noted that the theory and methodology, not to mention the language of systematics, have evolved considerably during the past two decades. Thus, it is difficult to interpret the precise meaning of earlier practitioners when they used terms such as "natural" to describe particular groupings, or designated particular elements of a major grouping as the group's "core." The situation is made more complex by the fact that similar statements concerning relationships among groups can mask differing opinions regarding the precise membership of each group.

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² Author for correspondence.

With these points in mind, we review the current situation, with the comprehensive system of Clayton and Renvoize (1986) as a reference point for discussion.

Four subfamilies (Pooideae, Bambusoideae, Chloridoideae, and Panicoideae) are among the more clearly delimited groupings of grasses recognized today (Clayton and Renvoize, 1986). Each includes a distinct "core" group of genera that share a set of putative synapomorphies, and with the core of each subfamily is associated a group of genera of questionable affinity. The Pooideae include most grasses of cool temperate to boreal regions (Stebbins, 1956, 1987; Stebbins and Crampton, 1961; Macfarlane and Watson, 1980, 1982; Macfarlane, 1987; Watson and Dallwitz, 1992). Several putative synapomorphies of the core Pooideae support their monophyly, and thus render the group an unlikely point of ancestry for other groups of grasses (e.g., Kellogg and Campbell, 1987; Macfarlane, 1987), and there is evidence that the Pooideae represent an early-diverged lineage within the family (Stebbins, 1982; Clayton and Renvoize, 1986; Kellogg and Campbell, 1987). *Brachypodium* (Table 1; included in Triticeae by Clayton and Renvoize [1986], segregated to a tribe Brachypodieae by other authors) and Meliceae are often regarded as elements of the "core" Pooideae, despite the occurrence in these taxa of anomalous characteristics, some shared with elements of other subfamilies (Stebbins, 1956; Tateoka, 1957a, 1960; Stebbins and Crampton, 1961; Watson and Dallwitz, 1992). Two other anomalous taxa, *Ampelodesmos* (constituting tribe Ampelodesmeae) and Stipeae, are included within the Pooideae by some authors and excluded by others (e.g., see Stebbins, 1956, 1982; Stebbins and Crampton, 1961; Decker, 1964b; Tsvelev, 1983; Dahlgren, Clifford, and Yeo, 1985; Clayton and Renvoize, 1986; Barkworth and Everett, 1987; Macfarlane, 1987; Watson and Dallwitz, 1992); elements of both of these tribes exhibit three lodicules and small chromosomes with x greater than 7, which are anomalous among the core Pooideae. Cladistic analyses have linked Meliceae and Stipeae with the core Pooideae (Kellogg and Campbell, 1987; Soreng, Davis, and Doyle, 1990), and have resolved a clade within the Pooideae that includes Bromaceae and Triticeae (Soreng, Davis, and Doyle, 1990; Kellogg, 1992); the latter two tribes, along with Brachypodieae, have been recognized as constituting the supertribe Triticoideae (Macfarlane and Watson, 1980, 1982; Macfarlane, 1987).

A second major grass subfamily, Bambusoideae, includes the distinct and apparently monophyletic woody bamboos (Soderstrom and Ellis, 1987). Several herbaceous genera, principally occupying mesic, montane habitats in tropical latitudes, are linked to the bamboos by a set of micromorphological, anatomical, cytological, and chemical features, and are recognized most frequently as one or more tribes within a broadly defined Bambusoideae (Tateoka, 1957a; Soderstrom and Calderón, 1974, 1979; Calderón and Soderstrom, 1980; Soderstrom, 1981a; Clayton and Renvoize, 1986; Soderstrom and Ellis, 1987; Tucker, 1988; Paisooksantivatana and Pohl, 1992). Among these allied herbaceous tribes are *Oryza* and its closest relatives, collectively tribe Oryzoae. When excluded from Bambusoideae, the Oryzoae usually are recognized as the core of a separate subfamily Oryzoideae, which itself is delimited narrowly by some authorities, so as to include

only the Oryzoae, and more broadly by others to include additional elements of the herbaceous Bambusoideae. In the latter case, taxa excluded from Bambusoideae and Oryzoideae have been recognized at times as constituting additional subfamilies. Thus, the woody bamboos constitute the "core" of the Bambusoideae, and competing taxonomic systems assign various numbers of herbaceous genera to this subfamily or to one or more small subfamilies that are in most cases interpreted as closely related to the bamboos. One of several competing hypotheses concerning higher-level phylogenetic relationships among grasses proposes an early diversification, principally in tropical forest environments, among forms that would be recognized as the herbaceous and woody Bambusoideae, with the other major grass lineages derived from among these elements (Bews, 1929; Tateoka, 1957a). Thus, the question of monophyly of the Bambusoideae/Oryzoideae complex is linked to questions concerning the deepest branches in the grass family.

Two other major groups of grasses, the Chloridoideae and Panicoideae, are most diverse in warm tropical and subtropical regions. Most species in these two subfamilies exhibit C4 photosynthesis, which occurs only rarely elsewhere in the family. Both of these subfamilies are recognizable on the basis of putative synapomorphies that make each of them, like Pooideae, an unlikely progenitor of any other major clade of grasses (Stebbins, 1982; Kellogg and Campbell, 1987), although there is evidence that the Chloridoideae may be polyphyletic (Jacobs, 1987; Hilu and Esen, 1993). The Chloridoideae are linked by various characters to a disparate assemblage of genera that are usually recognized as subfamily Arundinoideae (Jacobs, 1987; Watson and Dallwitz, 1992; Hilu and Esen, 1993), which supports suggestions that the Chloridoideae arose from among elements of the Arundinoideae (e.g., Stebbins, 1956; Tateoka, 1957a; Conert, 1987; Kellogg and Campbell, 1987); if so, the Arundinoideae would not be monophyletic. Additional complexity is added to this situation by the presence of a small subfamily, Centothecoideae, which also appears to be closely related to Arundinoideae, and the various interconnections among these elements have given rise to a general hypothesis that these four subfamilies (Panicoideae, Arundinoideae, Chloridoideae, and Centothecoideae) represent a closely related complex (Clayton and Renvoize, 1986; Hilu and Esen, 1988; but see Watson and Dallwitz, 1992), which we designate the "PACC" group.

Any hypothesis concerning relationships among multispecies taxa is contingent, of course, upon the precise membership of each of these taxa. Many grass genera that appear to combine characteristics typical of two or more subfamilies have been placed in different groupings by different authors, and these various placements complicate discussions of relationships among the major groupings. One of the more frequently observed syndromes (e.g., in *Ampelodesmos*, *Brachyelytrum*, *Diarrhena*, *Nardus*, and Stipeae) is the co-occurrence of attributes considered typical of Bambusoideae and Pooideae (Stebbins, 1956; Reeder, 1957, 1962; Tateoka, 1957b; Stebbins and Crampton, 1961; Schwab, 1972; Macfarlane and Watson, 1980; Campbell, Garwood, and Specht, 1986; Barkworth and Everett, 1987; Brandenburg, Estes, and Collins, 1991; Watson and Dallwitz, 1992). If either the Bambusoideae

TABLE 1. Species of *Joinvillea* and Poaceae sampled for chloroplast DNA restriction site variation; grass species arranged according to system of Clayton and Renvoize (1986).

Family Subfamily Tribe Species	Source of material and accession number*
Joinvilleaceae	
<i>Joinvillea ascendens</i> Gaudich.	NTBG-800379
Poaceae	
Arundinoideae	
Arundineae	
<i>Danthonia californica</i> (L.) P. Beauv.	PI-232247
<i>Phragmites australis</i> (Cav.) Steudel	Soreng 3884
Bambusoideae	
Bambuseae	
<i>Bambusa glaucescens</i> (Willd.) Holttum	BHC
<i>Pseudosasa japonica</i> (Siebold & Zucc.) Makino	BHC
Brachyelytreae	
<i>Brachyelytrum erectum</i> (Screber) P. Beauv.	Soreng 3427
Diarrheneae	
<i>Diarrhena obovata</i> (Gleason) Brandenburg	seed from Tiedye 5186 (DAO)
Nardeae	
<i>Nardus stricta</i> L.	BBG: seed from Royl & Schiers s.n., 1988, Hempel s.n., 1987
Olyreae	
<i>Lithachne humilis</i> Soderstrom	BHC from USZ
Oryzeae	
<i>Leersia virginica</i> Willd.	Soreng 3399
<i>Oryza sativa</i> L.	L (Hiratsuka et al., 1989)
Phareae	
<i>Pharus latifolius</i> L.	BHC from USZ
Centothecoideae	
Centotheceae	
<i>Chasmanthium latifolium</i> (Michaux) Yates	C
Chloridoideae	
Eragrostideae	
<i>Eragrostis curvula</i> (Schrader) Nees [as <i>Stipagrostis uniplumis</i>]	PI-365034
Panicoidaeae	
Andropogoneae	
<i>Miscanthus sinensis</i> Andersson cv. Gracillimus	C
<i>Zea mays</i> L. cv. Breeder's Choice	Soreng 3926 (all except <i>Pvu</i> II)
<i>Zea mays</i> L.	L (Larrinua et al., 1983); <i>Pvu</i> II
Paniceae	
<i>Pennisetum alopecuroides</i> (L.) Sprengel	C
Pooideae	
Aveneae	
<i>Arrhenatherum elatius</i> (L.) J. S. Presl	Davis & Soreng s.n.
<i>Deschampsia cespitosa</i> (L.) P. Beauv.	PI-311043
<i>Koeleria cristata</i> (L.) Pers.	PI-477978
Bromeae	
<i>Bromus inermis</i> Leysser	Soreng 3428 (<i>Bam</i> H I, <i>Hind</i> III, <i>Pst</i> I); PI-314071 (<i>Kpn</i> I, <i>Pvu</i> II, <i>Sal</i> I, <i>Sma</i> I)
Meliceae	
<i>Glyceria striata</i> (Lam.) A. Hitchc.	Davis & Soreng s.n.
<i>Melica altissima</i> L.	PI-325418
Poeae	
<i>Ampelodesmos mauritanicus</i> (Poiret) Durand & Schinz	Royl & Schiers s.n., 1988
<i>Dactylis glomerata</i> L. var. <i>glomerata</i>	Soreng 3430 (<i>Bam</i> H I, <i>Hind</i> III, <i>Pst</i> I); PI-311033 (<i>Kpn</i> I, <i>Pvu</i> II, <i>Sal</i> I, <i>Sma</i> I)
<i>Festuca arundinacea</i> Schreber	PI-304844
<i>Poa palustris</i> L.	Soreng 3354
<i>Sesleria insularis</i> (Sommier) ssp. <i>sillingeri</i> (Deyl) Deyl [as <i>S. elongata</i>]	PI-253719
Stipeae	
<i>Piptatherum miliaceum</i> (L.) Cosson [as <i>Oryzopsis miliacea</i>]	PI-284145
<i>Stipa barbata</i> E. Desv.	PI-229468
Triticeae	
<i>Brachypodium pinnatum</i> (L.) P. Beauv.	PI-440170

TABLE 1. Continued.

Family Subfamily Tribe Species	Source of material and accession number ^a
<i>Triticum aestivum</i> L. cv. Susquehanna	Soreng s.n. (all but <i>Sma</i> I)
<i>Triticum aestivum</i> L.	L (Ogihara and Tsunewaki, 1988: <i>Sma</i> I)

^a Sources of plant material or published data: BBG = Berlin Botanical Garden; BHC = Bailey Hortorium Conservatory; C = Cornell grounds; NTBG = National Tropical Botanical Garden; PI = USDA Plant Introduction Station; L = Literature, as cited. Vouchers deposited at BH unless otherwise stated.

or the Pooideae represents a paraphyletic assemblage whose constituent elements diverged near the base of the family, it should not be surprising if some genera are mosaics of apomorphies of one of these subfamilies in combination with plesiomorphies that usually occur in the other.

Previous attempts to resolve higher-level relationships among grasses with molecular characters usually have employed economically important genera such as *Saccharum*, *Sorghum*, and *Zea* (Panicoideae); *Oryza* (Bambusoideae/Oryzoideae); *Eleusine* (Chloridoideae); and *Avena*, *Hordeum*, and *Triticum* (Pooideae). One analysis of shared restriction fragments (Enomoto, Ogihara, and Tsunewaki, 1985) resolved the Pooideae and the Panicoideae, and placed a solitary representative of Chloridoideae marginally closer to Pooideae than to Panicoideae, with *Oryza* at a greater distance. Other analyses have placed Chloridoideae with Panicoideae (Esen and Hilu, 1989), and *Oryza* with Pooideae (Hilu and Johnson, 1991). Two phylogenetic analyses rooted with nongrass outgroups have favored a relatively close relationship between Panicoideae and *Oryza*, with Pooideae placed in a sister-group relationship to these two (Doebley et al., 1990; Hamby and Zimmer, 1992), and in the latter case with the woody bamboo *Arundinaria* placed as sister group to a clade that includes all other grasses.

Kellogg and Campbell's (1987) phylogenetic analysis of the Poaceae presented two sets of relationships, some of them mutually inconsistent. Both of their trees resolved an initial dichotomy between two clades, the first a broadly defined "Poid clade" with *Nardus* as sister group, the second a clade that includes all other grasses. Within the second clade, both trees also resolved four of the other subfamilies as monophyletic (Bambusoideae, Chloridoideae, Panicoideae, and Centothecoideae [as Centothecae]), with genera of Arundinoideae occupying virtually every possible position among these subfamilies. Relationships among these four subfamilies differed substantially between the two trees presented. The first tree resolved successively more inclusive groupings of these subfamilies as follow: Panicoideae + Centothecoideae; this group + Chloridoideae; and this group + Bambusoideae. In terms of gross structure, this pattern is largely compatible with a monophyletic PACC group (i.e., excluding Bambusoideae), from which it differs only by the exclusion of genera of Arundinoideae that diverge from the clade below the point of divergence of Bambusoideae. The second tree, again with genera of Arundinoideae scattered among the other four non-Poid subfamilies, resolved groupings among those subfamilies as follow: Bambusoideae + Panicoideae; and Centothecoideae + Chloridoideae. The close relationship between Bambu-

soideae and Panicoideae in this tree renders it inconsistent with a monophyletic PACC group (i.e., excluding Bambusoideae), no matter how the Arundinoideae are delimited, for neither Centothecoideae nor Chloridoideae is placed in a group that includes Panicoideae but excludes Bambusoideae. As is evident in differences between these two trees, and between them and relationships resolved by other authors, consensus regarding higher-level relationships in Poaceae has yet to emerge.

Cladistic analysis of chloroplast DNA restriction site variation has proven feasible in angiosperms at the family level and higher (e.g., Palmer et al., 1988; Jansen et al., 1990; Jansen, Michaels, and Palmer, 1991; Downie and Palmer, 1992a). As in any phylogenetic analysis, homology assessment is a crucial element of such a study. The chloroplast genomes of *Oryza* and other grasses differ from those of most other plants by the presence of three inverted regions (Palmer and Thompson, 1982; Howe, 1985; Quigley and Weil, 1985; Howe et al., 1988; Hiratsuka et al., 1989; Shimada and Sugiura, 1989, 1991; Downie and Palmer, 1992b; Doyle et al., 1992). A survey of monocots detected all three inversions in every grass examined, including representatives of all subfamilies (Doyle et al., 1992). Outside the Poaceae, the largest of the inversions (ca. 28 kb) was detected only in Restionaceae and in the monogeneric families Ecdiocolaceae (*Ecdiocola*) and Joinvilleaceae (*Joinvillea*). The second largest of the inversions, ca. 6 kb in length, and overlapping one endpoint of the largest inversion, was detected only in grasses and *Joinvillea*, and the smallest (ca. 0.2 to 2 kb) only in grasses. The occurrence of both of the larger inversions in *Joinvillea* substantiates previously reported evidence for a close relationship between this genus and Poaceae (Hallier, 1912; Stebbins, 1982, 1987; Dahlgren, Clifford, and Yeo, 1985; Campbell and Kellogg, 1987; Linder, 1987; Manning and Linder, 1990). Also, the near colinearity of the chloroplast genomes of Poaceae and Joinvilleaceae suggests *Joinvillea* as a useful outgroup for cladistic analysis of chloroplast DNA restriction site polymorphisms in grasses. The present study is the first cladistic analysis of molecular variation that includes representatives of all conventionally recognized grass subfamilies; several genera of controversial affinity also are included.

Assessment of the stability of groups resolved by cladistic analysis is an area of great interest and controversy (see citations in Davis, Frohlich, and Soreng, 1993; and Davis, in press). One approach is the employment of strict consensus trees, which are unambiguous in meaning when parsimony is the criterion of support: if a group occurs in the consensus tree, it occurs in all most-parsimonious trees, and thus is "unequivocally supported" by the data

starched by a 24-hr dark treatment; then fresh leaf material was harvested and lyophilized; 0.2 to 0.5 g of the lyophilized material was shredded in a coffee grinder prior to suspension in solvents and separation of the chloroplast-enriched fraction; and 2×CTAB with 1% polyvinylpyrrolidone and 2-mercaptoethanol was added to the pelleted chloroplast-enriched fraction after the cyclohexane rinse. Subsequent steps followed the Doyle and Doyle method.

Restriction digests with *Bam*H I, *Hind* III, *Kpn* I, *Pst* I, *Pvu* II, *Sal* I, and *Sma* I, and electrophoretic separation, were conducted as in Soreng, Davis, and Doyle (1990). Standards were prepared by combining *Hind* III and *Pst* I digests of lambda DNA, which yielded a ladder of 24 fragments ranging in size from 0.1 to 23 kb. Digested DNAs were transferred (Southern, 1975) to charged nylon membranes (Zeta-Probe GT [Bio-Rad], or Hybond N+ [Amersham]). Chloroplast probes and lambda DNA were labeled with ³²P by random-priming (Feinberg and Vogelstein, 1984) and hybridized to the membrane-bound DNA at 65 C in the hybridization solutions of Palmer (1986) or Tanksley and Bernatzky (1986). Membrane washes, exposure to X-ray film, and stripping were conducted as previously reported (Soreng, Davis, and Doyle, 1990).

Restriction sites were mapped for complete chloroplast genomes using a double-pass probing sequence. A total of eight large cloned fragments, ca. 10–21 kb, principally from *Pennisetum americanum* (Thomas et al., 1984) and *Triticum aestivum* (Bowman et al., 1981), plus one from *Vigna radiata* (MB3; Palmer, Singh, and Pillay, 1983), first were used to determine gross structure; then 34 fragments (ca. 1–4 kb) from the *Nicotiana tabacum* clone library (Sugiura et al., 1986) were used to fill gaps between the larger probes and, as necessary, to resolve the fine-scale structure with reference to the published *Oryza* cpDNA sequence (Hiratsuka et al., 1989). Using this approach more than 99% of the chloroplast sequence was probed, and fragments as small as 0.3 kb (sometimes those as small as 0.15 kb) were routinely mapped.

Inferred restriction site homologies were scored as binary characters (Table 2), with sites scored as “missing” for accessions in which homology could not be assessed reliably. With *Joinvillea ascendens* as the outgroup, cladistic analysis was conducted with *Hennig86* (Farris, 1988), using the command *mhennig** followed by *bb**, then *nelson* to generate a strict consensus tree. The *Hennig86* analysis was repeated with 100 random taxon-entry sequences, which were generated with the *spin* function of *Dada* (Nixon, 1993). *Clados* (Nixon, 1992) was used to examine cladograms and to generate a printed cladogram.

SCR was conducted through two complete rounds (removal of single characters and all pairwise combinations). With 155 informative characters in the complete data set (see below), this overall analysis involved 155 analyses with $n - 1$ characters and 11,935 analyses with $n - 2$ characters. Each subanalysis was conducted with *Hennig86*, with the same options as in the basic analysis (but using only one taxon-entry sequence) as invoked by DOS batch files. An automated implementation of SCR will be available in *Dada* (Nixon, 1993) by the time this paper appears [K. C. Nixon, personal communication].

A bootstrap analysis was run using *Paup* (Swofford,

1991), with 1,000 replicates conducted by heuristic search with two random taxon entry sequences per replicate, using options *tbr*, *collapse*, and *mulpars*.

RESULTS

A total of 328 restriction sites in the chloroplast genomes of *Joinvillea* and 31 accessions of Poaceae were mapped, of which 155 (47%) were cladistically informative (i.e., present in at least two accessions, and absent in at least two). Of the remaining sites, 132 (40%) were either present or absent in just one accession (i.e., uninformative and interpretable as nonhomoplasious autapomorphies), and 41 (13%) were present in all accessions. Within the informative subset of the data matrix (32 terminals scored for 155 characters = 4,806 potential data points; Table 2) 154 cells (3.1%) were scored as missing, signifying “state unknown” for observed fragment patterns that were regarded as ambiguous concerning the presence of a site in a terminal; 17 of these cases (11% of all missing-value scores) occurred in *Joinvillea*.

All 100 replicate cladistic analyses of the entire data set (based on different taxon-entry sequences) resolved eight most-parsimonious trees of 402 steps, consistency index 0.39, and retention index 0.61 (Kluge and Farris, 1969; Farris, 1989; autapomorphies excluded from all three calculations). One of these eight trees is depicted in Fig. 1, with annotations specifying the structure of the strict consensus tree and minimum character removal scores as determined by SCR. Groups with a character removal score of 1 exhibited bootstrap frequencies between 18 and 60 (average 40), those with a character removal score of 2 yielded bootstrap frequencies between 65 and 93 (average 79), and those with a character removal score ≥ 3 (no pairwise character combination sufficient to dissolve the group) yielded bootstrap frequencies between 62 and 100 (average 88).

The analysis resolved two major clades within the grasses (Fig. 1), one of which corresponds to a broadly defined Pooideae that includes *Ampelodesmos*, *Diarrhena*, and both of the sampled genera of Stipeae (*Piptatherum* + *Stipa*). Five major clades of mutually exclusive membership were resolved within the Pooideae: 1) *Ampelodesmos* + Stipeae; 2) *Diarrhena*; 3) *Brachypodium* + Meliceae (*Glyceria* + *Melica*); 4) *Bromus* + *Triticum*; and 5) Aveneae (*Arrhenatherum* + *Deschampsia* + *Koeleria*) + Poeae (*Dactylis* + *Festuca* + *Poa* + *Sesleria*). The analysis consistently resolved Aveneae + Poeae as sister group of *Bromus* + *Triticum*. Other relationships among these five clades varied among most-parsimonious trees: six of the trees resolved relationships as depicted in Fig. 1, with *Brachypodium* + Meliceae as sister group of the “core” Pooideae (*Bromus* + *Triticum* + Aveneae + Poeae); *Diarrhena* as sister group of this larger clade; and *Ampelodesmos* + Stipeae as sister group of all other Pooideae. The other two trees resolved a sister group relationship between the “core” Pooideae and a monophyletic assemblage of the other three groups. SCR revealed that the monophyletic grouping of Pooideae, including all of these elements, is robust to the removal of any single character or two-character combination, as are various groupings within the Pooideae, including Aveneae, Poeae + Ave-

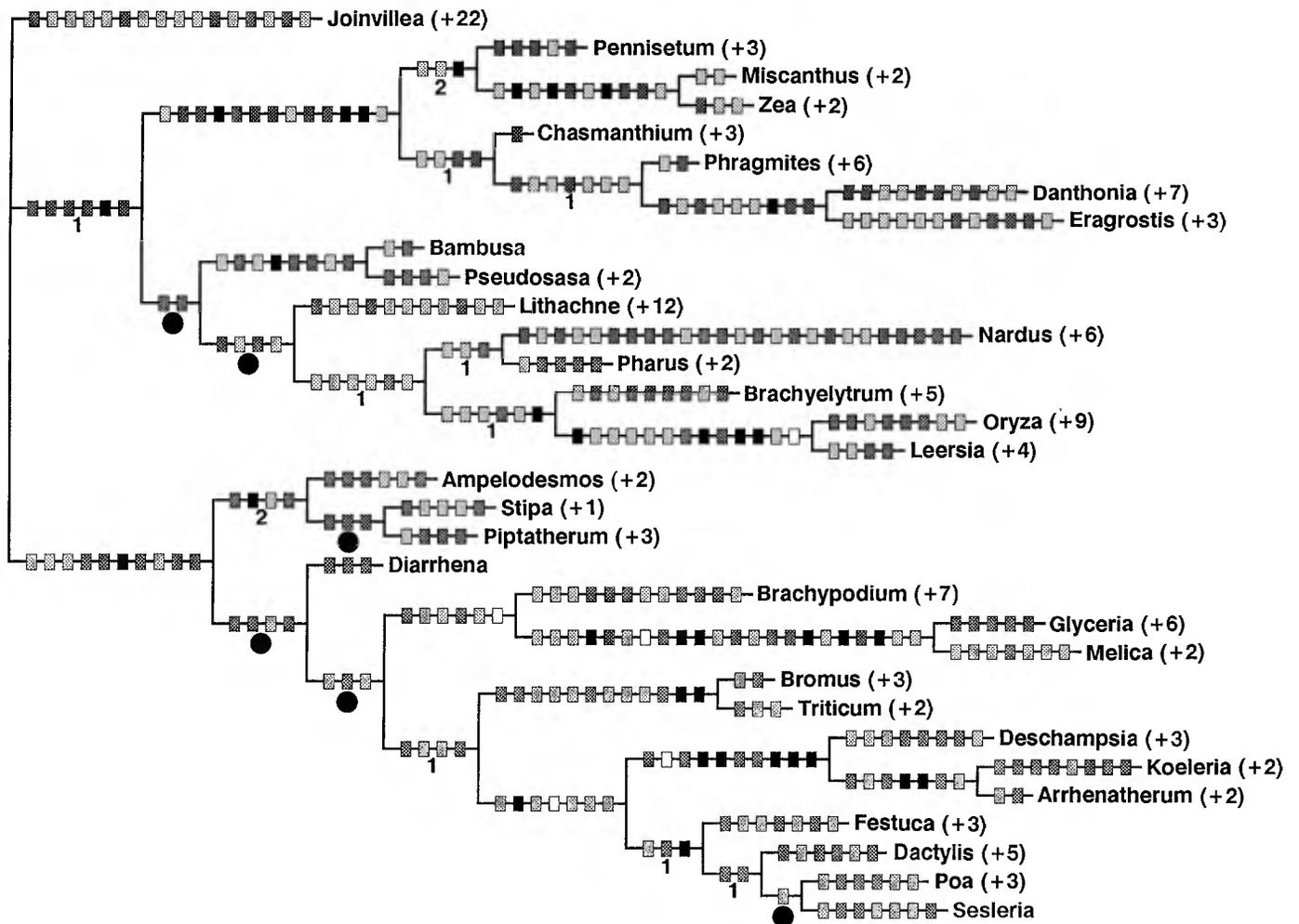


Fig. 1. One of eight most-parsimonious cladograms depicting relationships among chloroplast genomes of *Joinvillea* and 31 genera of grasses (Table 1), as resolved by 155 informative chloroplast DNA restriction sites (Table 2). Nonhomoplasious restriction site gains are depicted by black bars, losses by open bars; homoplasious gains are depicted with dark shading, losses by light shading; number of nonhomoplasious autapomorphies of each terminal is listed in parentheses following name of taxon; state changes between *Joinvillea* and Poaceae are mapped as "gains" or "losses" of *Joinvillea*. Closed circles mark clades that are absent from the strict consensus tree; numbers beneath branches are minimum character removal scores as determined by Successive Character Removal (see text); unmarked branches have scores ≥ 3 .

neae, *Bromus* + *Triticum*, and *Brachypodium* + Meliceae (Fig. 1).

The second major clade detected by the analysis comprises 1) Bambuseae (the woody bamboos, *Bambusa* + *Pseudosasa*); 2) most of the herbaceous genera that have been placed by various authors in Bambusoideae, Pooideae, or Oryzoideae (including *Brachyelytrum*, *Lithachne*, *Nardus*, *Pharus*, and *Oryzae* (*Leersia* + *Oryza*), but not *Diarrhena*); and 3) a monophyletic assemblage of Panicoideae (*Miscanthus* + *Pennisetum* + *Zea*), Arundinoideae (*Danthonia* and *Phragmites*), Centothecoideae (*Chasmanthium*), and Chloridoideae (*Eragrostis*), i.e., the PACC group or clade. Relationships within the PACC clade were resolved as a sister-group relationship between a monophyletic Panicoideae and a monophyletic grouping of all genera representing the other three subfamilies. Within the latter grouping, *Danthonia* (Arundinoideae) + *Eragrostis* (Chloridoideae) were resolved as a monophyletic group, and *Phragmites* (Arundinoideae) as the sister group of this pair.

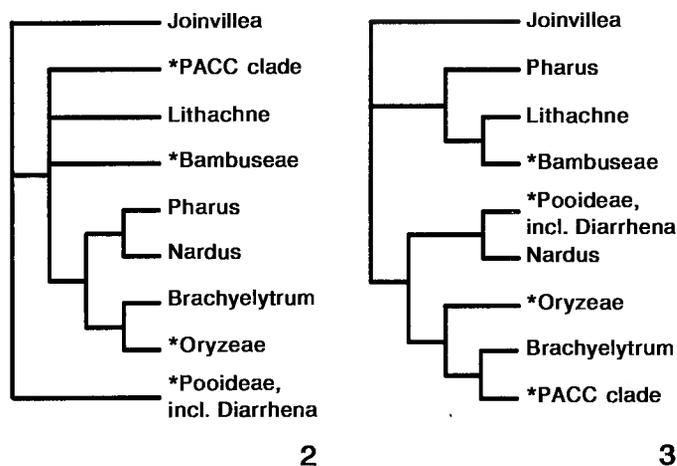
The remaining bambusoid/oryzoid elements of the PACC + Bambusoideae clade were resolved in two alternative topologies among the set of most-parsimonious trees. All eight trees resolved five genera of this complex (*Brachyelytrum*, *Nardus*, *Pharus*, and the two genera of Oryzoideae) as a monophyletic group. Four of the trees also resolved *Lithachne* and Bambuseae with these five in an inclusive monophyletic Bambusoideae, as sister group of the PACC clade (Fig. 1). The other four trees resolved a sister-group relationship between *Lithachne* and Bambuseae, and placed this pair of taxa as the sister-group of the PACC clade. The consensus of these structures (Figs. 1, 2) is an unresolved assemblage of four groups: the PACC clade, Bambuseae, *Lithachne*, and the grouping of *Brachyelytrum*, *Nardus*, *Pharus*, and Oryzoideae. SCR revealed that the most stable groupings in this major clade (character removal scores ≥ 3) are the PACC clade, two of the clades within it (*Miscanthus* + *Zea*, i.e., Andropogoneae; and *Danthonia* + *Eragrostis*), Bambuseae, and Oryzoideae. Except for the PACC clade itself, and the grouping of

Danthonia + *Eragrostis* within it, no group of elements representing different tribes of the Bambusoideae + PACC group is robust to the removal of two characters, nor is the entire group itself.

SCR analyses based on $n - 1$ (i.e., 154) characters usually resolved approximately the same monophyletic groups as did the complete data set, occasionally more or fewer. Among analyses that resolved groups (in consensus) that the complete data set did not, five resolved a monophyletic Bambusoideae (Bambuseae plus six herbaceous genera), as observed in four of the eight most-parsimonious trees obtained with the complete data set (i.e., as depicted in Fig. 1), and five resolved Bambuseae + *Lithachne* + the PACC clade, as in the other four most-parsimonious trees obtained with the complete data set. Seven single-character removals resulted in a loss of resolution in the strict consensus tree of the grouping of the PACC clade with Bambusoideae (i.e., Bambuseae, Oryzaceae, and four other herbaceous genera, as in Fig. 1). In all seven cases, the breakup of this group occurred in some most-parsimonious trees, but not all. An example is presented in Fig. 3, which depicts one of the sets of relationships resolved by the data set that lacks character 132 (Table 2). In this case, as with all trees resolved by the complete data set and by all possible data sets derived by the removal of one or two characters, a monophyletic Pooideae always was resolved in all most-parsimonious trees. Of these 53 trees, the monophyletic grouping of Bambusoideae plus the PACC group was resolved in 24; among the remaining 29, *Nardus* was resolved as sister group of the Pooideae in 23 (e.g., Fig. 3), while *Brachyelytrum* occupied this position in the other 6. When Bambusoideae + the PACC clade were not resolved as a monophyletic group, both the Pooideae and the PACC clade were always nested among elements of the Bambusoideae.

DISCUSSION

A major result of this analysis is the stable resolution of two principal clades in the grass family, the Pooideae and the PACC clade. These two clades include all grasses sampled except those of the Bambusoideae/Oryzoideae complex, which are resolved as allied with the PACC clade, although this alliance is unstable. Authors have differed substantially in their delimitations of Pooideae, and as noted above, strict monophyly has not always been a prime arbiter in these decisions. Several characters usually associated with grasses of other subfamilies (e.g., three lodicules, various embryonic characters, small chromosomes, and chromosome base numbers (x) of 9, 10, 11, or 12) occur in various combinations in *Ampelodesmos*, *Brachypodium*, *Diarrhena*, Meliceae, and Stipeae (Tateoka, 1960; Stebbins and Crampton, 1961; Decker, 1964b; Watson and Dallwitz, 1992), all of which are included in the monophyletic Pooideae resolved by the present analysis. In this respect our results are consistent with those of Kellogg and Campbell (1987), whose cladistic analysis of morphological characters also placed all five of these taxa in the Pooideae. Relationships among these taxa within the Pooideae remain unresolved, but none is ever placed among the tribes of what we have resolved as a monophyletic "core" Pooideae (i.e., Bromeae + Triticeae



Figs. 2, 3. Summary cladograms representing relationships among *Joinvillea*, four grass genera, and four groups of grass genera (marked with asterisks; see text for membership), as resolved by cladistic analysis of 155 informative chloroplast DNA restriction sites; relationships within groups of genera (marked by asterisks) are resolved to varying degrees but are not depicted, in order to emphasize higher level relationships in the family. 2. Strict consensus of eight most-parsimonious trees resolved by the complete data set. 3. One of 53 most-parsimonious trees resolved by data set lacking character 132 (see text and Table 2).

+ Aveneae + Poaceae). This structure suggests that anomalous characters exhibited by genera outside the "core" of the subfamily represent plesiomorphies of the subfamily as a whole. Within the "core" Pooideae, the grouping of Bromeae and Triticeae is not unexpected, but the sister-group relationship of this pair of tribes to the other pair—Aveneae and Poaceae—represents a clarification of relationships within this major temperate grass radiation. The grouping of *Brachypodium* with Meliceae conflicts with the more frequent placement of the former with Bromeae and Triticeae (e.g., Macfarlane and Watson, 1980, 1982; Macfarlane, 1987), and with the results of our previous analysis (Soreng, Davis, and Doyle, 1990); the affinities of *Brachypodium* warrant further attention.

Two notable taxa excluded from Pooideae by the present analysis are *Brachyelytrum* and *Nardus*. These genera often have been included within the Pooideae, and the analysis by Kellogg and Campbell (1987) resolved a "Pooide clade" that included *Brachyelytrum*, and placed *Nardus* as sister group of this clade. Because our analysis resolves the Pooideae (excluding both of these genera) as the sister group of a monophyletic assemblage of all other grasses sampled, no individual taxon outside the Pooideae is resolved as more closely related to the Pooideae than any other. The case for an affinity between *Brachyelytrum* and other Bambusoideae was made by Campbell, Garwood, and Specht (1986), and our results, by placing *Brachyelytrum* within a complex of bambusoid taxa, are consistent with such an affinity. As for *Nardus*, it is the only genus with bicellular microhairs (Scholz, 1982; Renvoize, 1985) that is frequently assigned to Pooideae. Hence, this character is equally consistent with the placement of *Nardus* as sister group of the Pooideae or at a more distant location in the family. One reason *Nardus* has been difficult to place is that it lacks lodicules, and hence all diagnostic

lodicle characters. Its placement in the present analysis with *Pharus*, which also lacks lodicules, suggests loss of lodicules as a synapomorphy of *Nardus* and Phareae, or provocatively, that lodicule presence may be a synapomorphy of all grasses except this group. We emphasize, however, that our SCR analysis indicates that there is support within the data set (albeit outweighed when all characters are included) for the placement of either *Brachyelytrum* or *Nardus* as sister group of the Pooideae (Fig. 3).

The second major clade resolved by this analysis is the PACC clade. As with the Pooideae, some relationships within this clade also are stable, notably a monophyletic Andropogoneae within a monophyletic Panicoideae. The PACC clade also includes a stable grouping of *Danthonia* + *Eragrostis*, to the exclusion of *Phragmites*, a relationship that is consistent with prevailing perceptions that the Arundinoideae are not monophyletic, and that the Chloridoideae are derived from among the Arundinoideae. The resolved relationship among these three genera is inconsistent not only with a monophyletic Arundinoideae, but with a monophyletic tribe Arundineae that includes both *Danthonia* and *Phragmites* (e.g., Clayton and Renvoize, 1986; Table 1). The present analysis therefore favors the segregation of *Danthonia* to tribe Danthoneae, as treated by various authors, but any solution along these lines is contingent upon careful evaluation of the generic limits of *Danthonia* and related genera (Conert, 1987).

As far as the present analysis is concerned, the stable resolution of Pooideae and the PACC clade leave as a remaining problem the placement of all other grasses relative to these two groups. Although our most-parsimonious trees place *Brachyelytrum* and *Nardus* within the Bambusoideae/Oryzoideae complex, in association with the PACC clade (Figs. 1, 2), this placement is not stable, and there is support in the data for the placement of either of these genera with Pooideae (Fig. 3). More general than this problem is the question of placement of all elements of the Bambusoideae/Oryzoideae complex, including *Brachyelytrum* and *Nardus*, plus *Lithachne*, *Pharus*, *Oryzae*, and the woody bamboos (Bambuseae). This entire group may be monophyletic, as resolved in some of our most-parsimonious trees (e.g., Fig. 1); if so, there would be three major clades within the grasses (Pooideae, Bambusoideae, and the PACC clade). Any possible sister-group relationship might then exist among these three clades, with our analysis, on balance, favoring a closer relationship between the latter two. Alternatively, the Bambusoideae sensu lato may not be monophyletic, in which case relationships among its various elements, relative to each other and to the Pooideae and the PACC clade, remain to be resolved. The monophyly of the Bambusoideae sensu lato, and relationships between its constituent taxa and the two major clades thus remain much in doubt.

Our findings are largely consistent with the conclusions of Clayton and Renvoize (1986), who recognized three principal radiations of grasses. One was the Pooideae, in which they included *Nardus*, but not *Diarrhena*. Their second group corresponds to a broadly defined Bambusoideae, with Bambuseae nested among various herbaceous taxa, including *Brachyelytrum*, *Diarrhena*, *Pharus*, *Oryzae*, and *Lithachne* (in Olyreae). Their third group

includes the remaining four subfamilies, and our PACC clade is congruent with that group in membership, and in the recognition of a major division within that group between the Panicoideae and the other three constituent subfamilies.

Doebley et al. (1990) sampled *Oryza* and several representatives each of Panicoideae and Pooideae, and resolved *Oryza* as sister group of a monophyletic Panicoideae, with a monophyletic Pooideae as sister of that larger group; our results are congruent with this set of relationships. The relationships detected by Hamby and Zimmer (1992) are congruent with those of Doebley et al. (1990), but Hamby and Zimmer also sampled *Arundinaria* (Bambuseae), and their analysis placed it as sister group of all other grasses sampled (i.e., separate from Oryzae). This resolution lends credence to the possibility that Bambusoideae sensu lato is not monophyletic, but it is inconsistent with the initial division we resolve between Pooideae and all other grasses. Neither of these analyses included elements of Arundinoideae, Chloridoideae, or Centothecoideae, so their resolution of a sister group relationship between Panicoideae and Oryzae does not speak to the question of a monophyletic PACC clade.

Kellogg and Campbell (1987) did include all subfamilies in their analysis, and our results are consistent with their resolution of an initial division between Pooideae and all other grasses. Our results differ from theirs on the question of whether *Nardus* is included in the Pooideae clade; although we resolve this relationship in some trees derived from data sets lacking single characters (e.g., Fig. 3), it does not occur among most-parsimonious trees derived from our complete data set. Kellogg and Campbell (1987) resolved a monophyletic Bambusoideae that includes both Bambuseae and Oryzae, a relationship that is inconsistent with those resolved by Hamby and Zimmer (1992), and one on which our data are not decisive. A conspicuous feature of Kellogg and Campbell's analysis is the widespread distribution of genera of Arundinoideae, among which are placed the Bambusoideae, Centothecoideae, Chloridoideae, and Panicoideae. Because our analysis includes only two genera of Arundinoideae, it may turn out that further sampling will place additional genera of this subfamily outside the currently resolved PACC clade, but the present sampling provides no supporting evidence for such a distribution. We turn, then, to the question of monophyly of the PACC clade, and of relationships among the non-Arundinoid elements within it. As noted above, Kellogg and Campbell presented two trees that resolved different relationships among these subfamilies. The first of their trees is largely consistent with the recognition of a PACC clade, from which it would exclude only those genera of Arundinoideae that diverge from this clade below the point of divergence of Bambusoideae. The structure they resolve within this group differs, however, from that which we resolve, most dramatically in their placement of *Danthonia* with Panicoideae and Centothecoideae (as Centothecoae), while our analysis places *Danthonia* with Chloridoideae in a group that excludes the other two subfamilies. Their second tree, which places Bambusoideae with Panicoideae in a monophyletic group that does not include Centothecoideae or Chloridoideae, is distinctly at odds with our resolution of a PACC clade. The placement of Bambusoideae relative to other grasses

thus remains a key to our understanding of the earliest diversification of grasses.

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