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A phylogenetic analyis of chloroplast DNA restriction site variation in *Poaceae* subfam. *Pooideae*

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Key words: Angiosperms, *Poaceae, Pooideae.* – Phylogenetics, evolution, chloroplast DNA, restriction site variation.

Abstract: A phylogenetic analysis was conducted on chloroplast DNA restriction site variation in 34 genera of grasses (family *Poaceae*), including 28 genera from subfam. *Pooideae* (representing tribes *Aveneae, Brachypodieae, Bromeae, Meliceae, Poeae, Stipeae,* and *Triticeae*) and representatives of three other subfamilies, *Arundinoideae, Oryzoideae,* and *Panicoideae.* Analyses of all 34 genera always distinguished *Pooideae* as monophyletic, regardless of which nonpooid genus functioned as outgroup; six separate analyses of all 28 pooid genera, each including one of the six nonpooid genera as outgroup, resolved five identically-constituted clades within *Pooideae* (in four cases), or (in the other two cases) yielded results that were less well resolved, but not in conflict with those of the other four analyses. The four best-resolved analyses distinguished *Meliceae* as the earliest diverging lineage within *Pooideae,* and *Stipeae* as the next. Above the point of divergence of *Stipeae* is a dichotomy between supertribe *Triticodae* (including tribes *Brachypodieae, Bromeae,* and *Triticeae*), and a clade comprising *Poeae* and *Aveneae.* The analysis supports some tribal realignments, specifically the assignment of *Briza, Chascolytrum, Microbriza,* and *Torreyochloa* to *Aveneae,* and *Arctagrostis, Catabrosa,* and *Sesleria* to *Poeae.* The analysis also suggests that the pooid spikelet (i.e., glumes shorter than lemmas and florets two or more) is plesiomorphic in *Pooideae,* and that spikelets with one floret, and those with glumes longer than the first lemma, each have evolved more than once within *Pooideae.* Results also indicate that small chromosomes and chromosome numbers based on x = c. 10 – 12 are plesiomorphic within *Pooideae.* Alternative states of these characters (chromosomes large, chromosome numbers based on x = 7) are interpreted as synapomorphies or parallelisms of clades that include *Triticodae, Aveneae,* and *Poeae.* Lanceolate lodicule shape may be a synapomorphy of the clade that includes *Stipeae, Triticodae, Aveneae,* and *Poeae,* and loss of lodicule vascularization a synapomorphy of the entire *Pooideae.*

Major syntheses of morphological, anatomical, cytological, and physiological data resolved the principal subdivisions of the grass family by the early 1960s (TATEOKA 1957, STEBBINS & CRAMPTON 1961). Six subfamilies usually are recognized today: *Arundinoideae, Bambusoideae, Chloridoideae, Oryzoideae, Panicoideae,* and *Pooideae* (STEBBINS & CRAMPTON 1961), although slightly different treatments also have been proposed (e.g., that of CLAYTON & RENVOIZE 1986). During the past 30 years, a time of considerable research activity (cf. SODERSTROM & al. 1987), interpretation

of boundaries between the subfamilies and tribes has remained fairly stable, but phylogenetic patterns among these taxa, particularly among tribes of the *Pooideae*, remain obscure. Phenetic (MACFARLANE & WATSON 1980, HILU & WRIGHT 1981, WATSON & al. 1985) and cladistic (BAUM 1987; KELLOGG & CAMPBELL 1987; E. A. KELLOGG, unpubl.) analyses bearing on the *Pooideae* have not resolved the subfamily's phylogenetic structure. This problem is frequently attributed, at least in part, to the simplified morphology of grasses: against a relatively uniform background, minor variants assume disproportionate significance, and even a few parallelisms can obscure phylogenetic patterns.

Among major phylogenetic questions involving the *Pooideae* are their relationship to other subfamilies, and the inclusion of tribes *Meliceae* and *Stipeae*, which generally are interpreted as loosely allied to the more homogeneous core *Pooideae* (STEBBINS 1956, TATEOKA 1957, MACFARLANE & WATSON 1980, TSVELEV 1983, CLAYTON & RENVOIZE 1986, BAUM 1987, KELLOGG & CAMPBELL 1987, MACFARLANE 1987). There is also a need to delimit monophyletic tribes within the core *Pooideae*. Specific problems in this area include characterization of the *Poeae* and *Aveneae* (including *Agrostideae*; HILU & WRIGHT 1982, MACFARLANE 1987), and clarification of the relationships of supertribe *Triticodae* (including tribes *Triticeae, Bromeae,* and *Brachypodieae*; MACFARLANE & WATSON 1982, MACFARLANE 1987). At another level, tribal assignment of several genera remains in question. Affinities of some controversial genera (e.g., *Lolium*) have been resolved, or were thought to have been resolved (e.g., *Torreyochloa*; CHURCH 1949, 1952; CLAUSEN 1952), while the appropriate placement of other genera (e.g., *Arctagrostis, Brachypodium, Catabrosa, Cinna, Koeleria,* and *Phippsia*) remains uncertain.

Molecular analyses of higher level phylogenetic relationships, in conjunction with critical interpretation of the homology of traditional characters, have great potential for resolving these problems. Previous molecular studies of higher level relationships in *Poaceae* include sequencing analyses of 18 S and 26 S ribosomal RNA (HAMBY & ZIMMER 1988), and of the large subunit of ribulose 1,5-bisphosphate carboxylase (rbc L) (ZURAWSKI & al. 1984; CLEGG & al., unpubl., cited in PALMER & al. 1988); and phenetic analyses of chloroplast DNA (cpDNA) restriction fragment length polymorphisms (RFLP) (VEDEL & al. 1980, ENOMOTO & al. 1985, LEHVÄSHLAIHO & al. 1987). These studies support the traditional taxonomic structure of the grass family but small sample sizes (11 or fewer genera), and in some cases the nature of the analytical techniques employed, have prevented the detailed resolution of phylogenetic pattern (PALMER & al. 1988).

Here we examine the cladistic structure of *Pooideae*, focusing on the questions discussed above. Each of the major tribes of *Pooideae*, as delimited by CLAYTON & RENVOIZE (1986), is represented in our study: *Aveneae, Bromeae, Meliceae, Poeae, Stipeae,* and *Triticeae*. We also include two tribes that are often recognized by other authors, *Brachypodieae* and *Seslerieae*. Not included in our sample are *Hainardieae* (five monotypic genera, and one genus of six species) and the monotypic tribes *Brylkinieae, Lygeae,* and *Nardeae*. We present new cpDNA restriction site (RS) data for 24 genera, and have produced RS maps of each genus for seven enzymes. We compare these data with available cpDNA maps of other genera from subfamilies *Arundinoideae, Oryzoideae, Panicoideae,* and *Pooideae*. Our results support inclusion of *Meliceae* and *Stipeae* within *Pooideae*, as early-diverging elements. Within *Triticodae,* the *Brachypodieae* tentatively are identified as sister group of

the *Bromeae/Triticeae*. The *Aveneae* either are: (1) derived from within a paraphyletic *Poeae* (including *Seslerieae*); or (2) assuming that intertribal transfers of chloroplast genomes have not occurred, the sister group of *Poeae*, if generic realignments are made in accordance with our results.

Materials and methods

Purified cpDNA was isolated from 33 species (Table 1, voucher specimens at BH) using minor modifications of DALLY & SECOND's (1989) organic solvent extraction procedure. Mature plants or seedlings were destarched by placement in a dark chamber for 12 h to 2 d, and fresh leaves were harvested and quick frozen in liquid N_2 or in a $-80\,^{\circ}C$ freezer, and then lyophilized. Prior to homogenization, leaves were shredded by hand or cut with scissors to reduce fragment size. Chloroplasts were extracted from 0.5 – 1.5 g dry mass (about 10% of fresh mass) of leaf material. We followed DALLY & SECOND's (1989) method for subsequent steps, except that we increased the concentration of Triton-X-100 2 – 3-fold for lysing organelle membranes, extracted the cpDNA once with phenol : chloroform (1 : 1) and once with chloroform : isoamyl alcohol (24 : 1), treated it with RNase *A*, and precipitated it once with isopropanol and once with ethanol; the extraction yielded 3 – 25 µg of cpDNA.

Restriction digests were performed according to the manufacturer's instructions (BRL) using seven enzymes that recognize unique six-base-pair sequences: HindIII, HpaI, KpnI, PstI, PvuII, SalI, and SmaI. Because of difficulties encountered previously in the digestion of grass cpDNA we used 20 to 25 units of restriction enzymes per µg of cpDNA to insure complete digestion. Digests were size-fractionated electrophoretically in DNA grade agarose (1% for HindIII, 0.5% for other enzymes). Ultraviolet illuminated restriction patterns were photographed, and SOUTHERN (1975) transfers were made to Zetaprobe nylon filters (Bio-Rad). Clones from a *Pennisetum americanum* cpDNA recombinant library (THOMAS & al. 1984) were labeled with ^{32}P by nick-translation (MANIATIS & al. 1982) or random-priming (FEINBERG & VOGELSTEIN 1984), and hybridized to the filter-bound cpDNA (PALMER 1986). The filters were exposed to X-ray film (Kodak XAR-5), then stripped for re-hybridization (PALMER 1986).

Restriction digest patterns were diagrammed from the UV photographs, and fragment sizes measured. The general order of single digest fragments was indicated by their homology with specific *Pennisetum* cpDNA probes. Fragments hybridizing to adjacent probes, and subsequent restriction site changes among cpDNA fragments of different species within probe regions, were used to arrange fragments linearly for each enzyme. The entire chloroplast genome was mapped in this manner for 30 species (Table 2) with each enzyme except HindIII. For HindIII digests we mapped only the small single-copy (SSC) region and the inverted-repeat (IR) regions. To screen for intrageneric polymorphism in restriction sites defining major clades, two species each of *Arctagrostis, Briza, Chascolytrum, Glyceria, Leucopoa, Melica, Puccinellia*, and *Torreychloa* were examined. In the largest grass genus, *Poa*, 44 species have been examined (SORENG 1990).

The *Pennisetum* clone library covers c. 91% of the chloroplast genome. For our mapping we used six fragments (pMCS 1, 4, 8, pMCSp 5, 6, and pMCP 1) ranging in size from 13 to 21 kb (THOMAS & al. 1984). This library is incomplete for a 12 kb region between the junction of the IR at psbA and psbD in the large single-copy region (LSC), but because this includes part of the grass inversion region (HOWE & al. 1988) we were hesitant to use probes of species that lack these inversions. In addition, a 7.5 kb clone from within the IR was not used. However, by working with purified cpDNAs digested in quantities sufficient to resolve fragments as small as 0.4 kb consistently via UV photography, and with enzymes that tend to produce rather large fragments, sufficient overlap of arrangements into adjacent probe regions or additivity and intensity of unprobed fragments allowed us to identify and arrange nearly all fragments and to develop internally consistent restriction site maps.

Table 1. Species of *Poaceae* included in the present study, arranged by subfamily and tribe, and sources of plant material and published data. Source codes denote origins of live material for new data generated in this study (voucher specimens at BH), and literature citations for published data (*a* map data, *b* fragment data, *c* sequence data): *INTA* Instituto de Botanica Agricola, Argentina; *JID* J. I. DAVIS collection; *NPI* Native Plants Inc., Utah; *RJS* R. J. SORENG collection; *USDA* USDA Plant Introduction Station (acquisition name in parentheses when identity of voucher does not agree with distribution name). * Tribal realignment suggested by this study

Species	Source
Arundinoideae	
Aristideae	
1. *Stipagrostis uniplumis* (LICHTENST.) DE WINTER	USDA 365034
Arundineae	
2. *Danthonia californica* (L.) BEAUV.	USDA 232247
Oryzoideae	
Oryzeae	
3. *Oryza sativa* L.	HIRAI & al. (1985) (a); HIRATSUKA & al. (1989) (c)
Panicoideae	
Andropogoneae	
4. *Sorghum bicolor* (L.) MOENCH	DANG & PRING (1986) (a)
5. *Zea mays* L.	LARRINUA & al. (1983) (a)
Paniceae	
6. *Pennisetum americanum* (L.) SCHUM.	THOMAS & al. (1984) (a)
Pooideae	
Aveneae	
7. *Arrhenatherum elatius* (L.) BEAUV. ex J. PRESL	JID & RJS s.n.
8. *Avena sativa* L.	ENOMOTO & al. (1985) (b)
9. *Deschampsia cespitosa* BEAUV.	USDA 311043
10. **Briza media* L.	USDA 442451
11. **Briza minor* L.	USDA 378653
12. **Chascolytrum erectum* (LAM.) DESV. (*Briza erecta*)	USDA 282880
13. **Chascolytrum subaristata* (LAM.) DESV. (*Briza subaristata*)	USDA 312826
14. **Microbriza poaemorpha* (J. S. PRESL) NICORA & RÚGOLO (*Briza poaemorpha*)	USDA 353466
15. **Torreyochloa erecta* (A. HITCHC.) CHURCH	RJS 3375
16. **Torreyochloa pauciflora* (J. S. PRESL) CHURCH	JID 533
Brachypodieae	
17. *Brachypodium pinnatum* (L.) BEAUV.	USDA 440170
Bromeae	
18. *Bromus inermis* LEYSSER	USDA 314071
Meliceae	
19. *Glyceria grandis* S. WATSON	JID & RJS s.n.
20. *Glyceria striata* (LAM.) A. HITCHC.	JID & RJS s.n.
21. *Melica altissima* L.	USDA 325418

Table 1 (continued)

Species	Source
22. *Melica cupanii* GUSS.	USDA 383702
23. *Schizachne purpurascens* (TORREY) SWALLEN	RJS & al. 3348
Poeae	
24. **Arctagrostis latifolia* (R. BR.) GRISEB.	USDA 372661
25. **Arctagrostis poaeoides* NASH ex BRITTON & RYDB.	USDA 372662
26. *Bellardiochloa violacea* (BELLARDI) CHIOV. (*Poa araratica*)	USDA 353455
27. **Catabrosa aquatica* (L.) BEAUV.	JID 515
28. *Dactylis glomerata* L.	USDA 311033
29. *Festuca arundinacea* SCHREBER	USDA 304844
30. *Festuca pratensis* HUDSON	LEHVÄSHLAIHO & al. (1987) (b)
31. *Leucopoa karatavica* (BUNGE) KRECZ. & BOBROV	USDA 229499
32. *Leucopoa sclerophylla* (BOISS. & HOHEN.) KRECZ. & BOBROV	USDA 275336
33. *Lolium multiflorum* LAM.	LEHVÄSHLAIHO & al. (1987) (b)
34. *Poa lanigera* NEES	INTA
35. *Poa palustris* L.	RJS 3354
36. *Puccinellia distans* (L.) PARL. cv. 'fults'	NPI PUDI 5591
37. *Puccinellia gigantea* (GROSSH.) GROSSH.	USDA 384944
38. *Sclerochloa dura* (L.) BEAUV.	R. MARTIN s.n.
39. **Sesleria coerulea* (L.) ARD. (*S. elongata*)	USDA 253719
Stipeae	
40. *Piptatherum miliaceum* (L.) COSSON (*Oryzopsis miliacea*)	USDA 284145
41. *Stipa barbata* E. DESV.	USDA 229468
Triticeae	
42. *Hordeum vulgare* L.	POULSEN (1983) (a); ENOMOTO & al. (1985) (b)
43. *Secale cereale* L.	ENOMOTO & al. (1985) (b)
44. *Triticum aestivum* L.	BOWMAN & al. (1981) (a); ENOMOTO & al. (1985) (b)

For comparisons between species, restriction site maps for each enzyme were aligned and calibrated from 0 – 135 kb, beginning from the middle of the SSC and proceeding in the shortest direction toward rbcL in the LSC (Fig. 1). Also, double digest maps of *Hordeum vulgare* (POULSEN 1983), *Triticum aestivum* (BOWMAN & al. 1981), *Oryza sativa* (HIRAI & al. 1985), *Pennisetum americanum* (THOMAS & al. 1984) *Poa* spp. (SORENG, unpubl.), *Zea mays* (LARRINUA & al. 1983), and *Sorghum bicolor* (DANG & PRING 1986), and the cpDNA sequence of *Oryza sativa* (HIRATSUKA & al. 1989), were aligned with our maps. Where perfect alignment of restriction sites was prevented by addition and deletion events or by variation in fragment size apparently arising from different investigators' measurements, we inferred site homology on the basis of proximity and presence/absence of associated changes in lengths of adjacent fragments. It was also possible to make tentative alignments of fragments of unmapped KpnI, PstI, and SalI digests of *Avena sativa, Secale cereale*, and *Triticum aestivum* (ENOMOTO & al. 1985), and of PvuII digests of *Festuca pratensis* and *Lolium multiflorum* (LEHVÄSHLAIHO & al. 1987), where they differed little or not at all from mapped fragment patterns of closely related species or genera.

Shared restriction sites were scored as presence/absence data and analyzed cladistically (under global parsimony) with HENNIG 86 (FARRIS 1988) by implicit enumeration of all possible trees. A shortest tree using *Danthonia* as the outgroup was analyzed with CLADOS (K. C. NIXON, unpubl.) for the distribution and consistency of restriction site changes, to investigate alternative trees, and to print cladograms. Putative restriction site gain and loss events were positioned on the cladogram to minimize the number of parallel gain and loss/gain events (DEBRY & SLADE 1985, TEMPLETON 1983). Alternative cladograms produced under Dollo parsimony were constructed with PHYLIP (FELSENSTEIN 1987).

Table 2. Shared restriction sites among grass species. Sites arranged by enzyme (cf. Fig. 1); species numbered as in Table 1, where sources of data are noted. Group 1 = species in Fig. 2; group 2 = additional species compared. For *Oryza* (taxon 3, group 2), 3 a = map data, 3 b = sequence data. Data codes: *0* restriction site absent; *1* present; – missing data; * presence possibly by parallel gain

Character numbers								
	1	2	3	4	5	6	7	8	9
	123456	78901234567	8901234567890	12345678901234567890	2345678901	2345678901234	567890123456789012
Restriction enzymes and map locations								
	Hind III	Hpa I	Kpn I	Pst I	Pvu II	Sal I	Sma I	
	1	1		11	11	1	11	
	12330	3445567880	334444677799	123555556778999900	44556700	23378999993	233445667778888803	
	514456	41686729596	8240249867813	06404014566192023403	0538685267	2773741456781	915278251341256871	
Possible parallel gains			*	*	*	** * *	*	*	*
Group 1								
2.	011110	01000011000	0001100011010	10000001000010001010	1101101001	0000001001100	100100010010000010	
7.	011011	00001011100	1010000101000	10001001001011000111	0000001100	0110011011100	000100001001101011	
9.	011011	00001011100	1010000101000	10001001001011000111	0000001100	1110011011100	000100001001101010	
11.	011011	00001011100	1010000101000	10001001001011000111	0000001100	0110011011100	000100001001101011	
12.	011011	00001011100	1010010101000	10001001001011000111	0000001100	1110011011100	000100001001101010	
15.	011011	00001011100	1010000101000	10001001001011000111	0000001110	0010011011100	000000001001101010	
17.	011011	00011011000	1011000110000	10001101000010000100	0000011001	0100011001100	100100100001000011	
18.	010011	00011001100	1011001101101	10000001000000000100	0000001101	0100011001110	100100100001000011	
19.	111011	00000001100	0111010110010	10001101100010001010	0000011000	0100011001100	010101100001000110	
20.	111011	00000001100	0011010110010	10001101100010001010	0010011000	0100011001100	010101100001000110	
21.	101011	00000101100	0111010110000	00001101100010001010	0010001000	0100010001100	010101100000000110	
22.	101011	00000101100	0111010110000	00001101100010001010	0010010000	0100010001100	010101100000000110	
25.	011011	00001001100	1010000101000	10001101000110000110	0000001100	0100111011100	000000000001000001	
26.	011011	00001001110	1000000101000	10001101000010000110	0000001100	0100111011100	000000000001000000	
27.	011011	00001001110	1010000001000	11001101000110000010	0000001100	0100011011100	000100000000010011	
28.	011011	00001001100	1010000101000	10001101000010000110	0000001100	0100111011100	000000000001000001	
29.	011001	00001001110	1010000101000	10001101000010000100	0000001100	0100111011100	001100000101000010	
32.	011001	00001001110	1010000101000	10001101000010000100	0000001100	0100111011100	001100000101000010	
34.	011011	10001001100	1010000101000	10001101010110000110	0010001101	0100111011100	000000000001000001	
35.	011011	10001000100	1010000101000	10001101010110000010	0000001101	0100111011100	000000000001000001	
36.	011011	00001001110	1010000001000	11001101000100000010	0000001100	0100011011100	000100000000010011	
38.	011011	00001001110	1010000001000	11001101000110000010	0000001100	0100011011100	000100000000010011	
39.	011011	00001001110	1010000101000	10001101000110000010	0000001000	0100111011100	000100000001000011	
40.	011011	00100011101	1011000111010	00000001000010000110	0001001000	0100011001100	000110100001000011	
41.	011011	00100011101	1011000111010	00000001000010000110	0000001000	0100001001100	000110100001000011	
42.	010011	----------	1010001101101	10000000000000000000	0000001101	0100010111000	----------	

Table 2 (continued)

Group 2							
1	011110	00010011000	1001100111000	10000000000010001010	1101101001	0000011011100	10001101001000010
3a.	------	----------	----------	10001001000010000010	0000101001	0000010010001	----------
3b.	011110	00001011000	1001000101110	10001001000010000010	0000101001	0000010011001	00010010000001010
4.	------	----------	----------	10110011000010011010	----------	0001011000111	----------
5.	------	----------	----------	10111011000010011010	0100100001	0001011000111	----------
6.	------	01000010000	----------	----------	----------	0000011001100	----------
8.	------	----------	1010000101000	10001001001011001011	----------	0110001011000	----------
10.	011011	00001011100	1010000101000	10001001001011000111	0000001100	0110011011100	00010000100110101 1
13.	011011	00001011100	1010010101000	10001001001011000111	0000001100	1110011011100	00010000100110101 0
14.	011011	----------	1010010101000	10001001001010000111	0000001100	1110011011100	00010000100110101 0
16.	011011	00001011100	1010000101000	10001001001011000111	0000001110	0010011011100	00000000100110101 0
23.	------	----------	0011010110000	00001101100010000010	0010011000	0100011001100	01010110000000011 0
24.	011011	00001001100	1010000101000	10001101000110000110	0000001100	0100111011100	00000000000100000 1
30.	------	----------	----------	----------	0000001100	----------	----------
31.	011001	00001001110	1010000101000	10001101000010000100	0000001100	0100111011100	00110000010100001 0
33.	------	----------	----------	----------	0000001100	----------	----------
37.	011011	00001001110	1010000001000	11001101000100000010	0000001100	0100011011100	00010000000010011
43.	------	----------	1010001101101	10000001000000100100	----------	0100011111000	----------
44.	------	----------	1010001101101	10000001000000100100	----------	0100011111000	----------

Results

Comparisons of RS patterns of 24 genera from our digests, five genera from published fragment data, six from published maps, and one genus from a published sequence, yielded our raw data set of RS presence/absence (Table 2). Reported sizes for the chloroplast genome vary from 130 to 141 kb among grasses, and estimates by different authors of variation within species vary (e.g., *Oryza sativa* by 6 kb, *Zea mays* by 2.4 kb). Our study detected an average chloroplast genome size of c. 135 kb in the *Pooideae*, close to the 134 525 bp of *Oryza* (HIRATSUKA & al. 1989), and a total range of less than 1 kb. Among genera sampled, the SSC was c. 13.6 kb, the LSC c. 77.4 kb, and each IR c. 22 kb (compare *Oryza:* SSC = 12.3 kb, LSC = 80.6 kb, IR = 20.8 kb; HIRATSUKA & al. 1989). Of 223 restriction sites characterized, 32 were in the IR; hence, 207 unique sites (c. 0.2% of the unique sequence of the chloroplast molecule) were identified. Of these 207 sites, 83 were phylogenetically informative in differentiating the *Pooideae* from *Danthonia*, or providing structure within *Pooideae*.

Informative restriction sites (putative synapomorphies) were more densely distributed in the LSC (0.97 sites/kb) than in the SSC (0.66 sites/kb) or IR (0.32 sites/kb) (Fig. 1). Regions of high variability were identified (Fig. 1). One such region was in the juncture of the IR and the LSC, near the psbA gene in grasses, a region of noted variability (PALMER 1983, MOON & WU 1988). A second hot spot was near map coordinates 93 – 95, near the endpoints of the 28 kb and c. 6 kb grass inversions (QUIGLEY & WEIL 1985). These inversion endpoints are known to contain repeated sequences (HOWE & al. 1988), and may be "hot spots" for RS change (ZURAWSKI & CLEGG 1987).

Gene arrangements appear to be essentially colinear among the *Panicoideae* and *Pooideae* (VEDEL & al. 1980, HOWE & al. 1988, QUIGLEY & WEIL 1985, SØGAARD

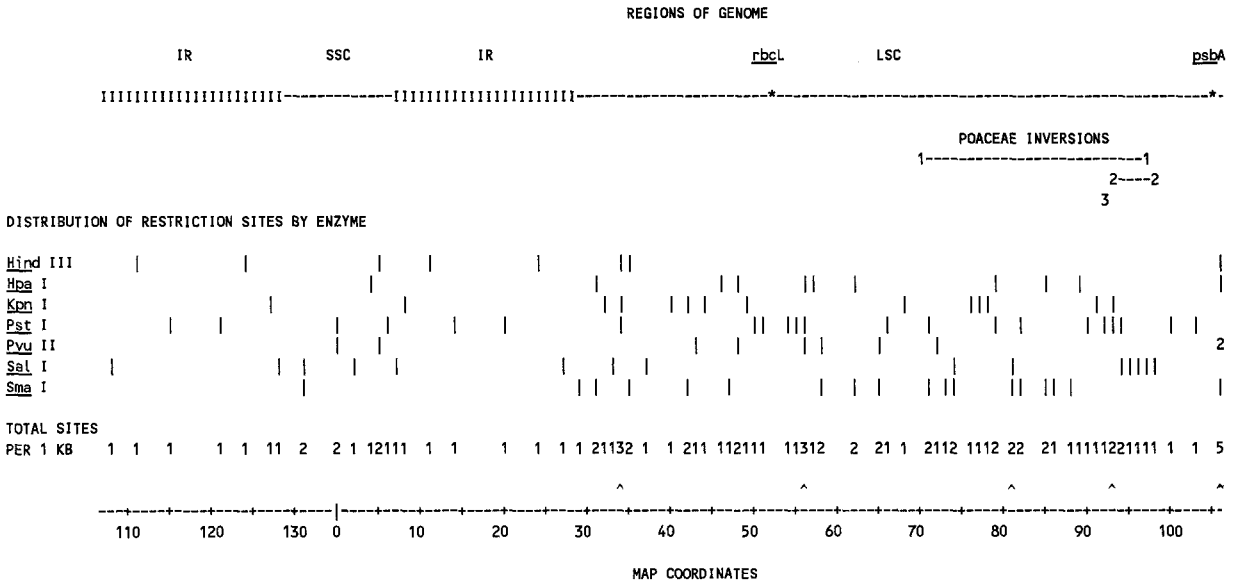

Fig. 1. Distribution of cladistically informative restriction sites (cf. Table 2) within 1 kb units of chloroplast genomes of the *Poaceae*. | restriction sites; −, **I** 1 kb units; ∧ possible "hot spots" of high restriction site variation. See text for discussion of the three inversions

a complete data set was available (*Danthonia*) as exemplar for the relationships *dinoideae* (our RS maps). All grasses so far examined in sufficient detail exhibit two large inversions and one small one (Fig. 1), none of which has been detected outside the family.

Cladistic analyses of the RS data, including *Danthonia, Oryza, Pennisetum, Sorghum, Stipagrostis,* and *Zea,* as well as the pooid genera, always resolved *Pooideae* as monophyletic, regardless of which of the six nonpooid genera served as outgroup. Six separate trial analyses also were run, each involving all of the pooid genera and a different one of the six nonpooid genera. Four of these analyses (those involving *Danthonia, Sorghum, Stipagrostis,* and *Zea*) resolved five identically constituted major groups within *Pooideae* (discussed below), and all most-parsimonious cladograms in these four cases resolved the same relationship among the five groups. When *Oryza* or *Pennisetum* was employed as the outgroup, resolution was diminished, presumably (with *Pennisetum*) because of the limited amount of data available, or (with *Oryza*) because accumulated RS differences between it and *Pooideae* diminish its ability to polarize characters in *Pooideae* (Table 2). Although the degree of resolution obtained with these two genera was less than that obtained with the other four, there were no inconsistencies in the relationships detected: clades resolved in these two analyses were subsets of those detected by the other four. Because *Danthonia, Sorghum, Stipagrostis,* and *Zea* yielded the greatest resolution, and independently resolved the same groups, we chose one of these genera for which a complete data set was available (*Danthonia*) as exemplar for the relationships discussed below.

Implicit enumeration of all possible solutions involving *Danthonia* and 25 species representing 22 genera of the *Pooideae* detected ten most-parsimonious cladograms

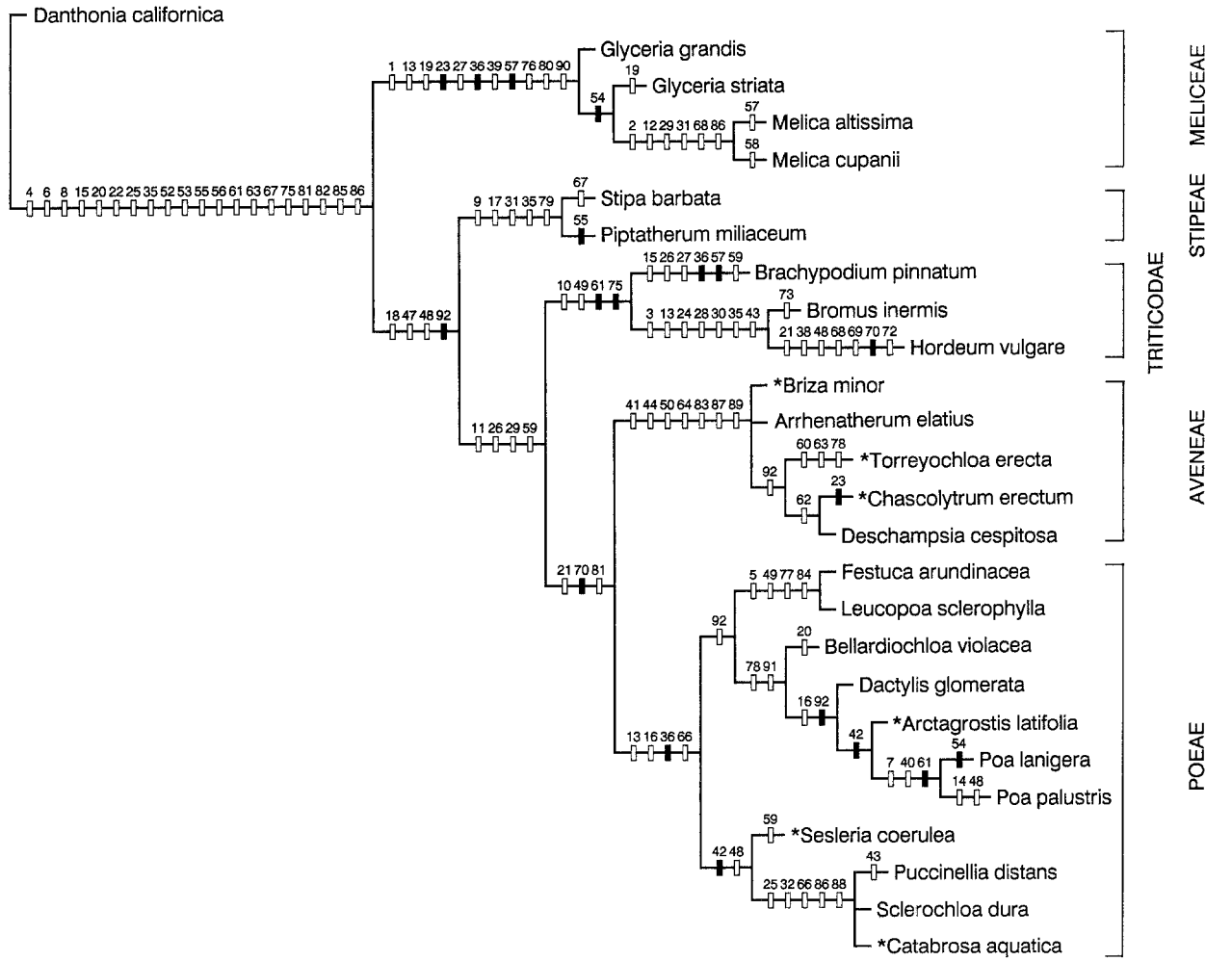

Fig. 2. One of ten most parsimonious cladograms of *Poaceae* subfam. *Pooideae* on the basis of cpDNA restriction site variation. Length = 120 steps (nonhomoplasious autapomorphies excluded), consistency index = 0.63. Numbers refer to restriction sites (Table 2). □ Site losses and unique site gains, ■ parallel site gains, including gain-loss-gain sequences (sites 55 and 75, present in *Danthonia,* represented as loss-gain sequences). Tribe and supertribe designations specify chloroplast types discussed in text; * tribal realignment suggested by the present study

of 127 steps and a consistency index of 0.63. Seven of the steps are autapomorphies of nonhomoplasious characters within data set 1 (Table 2); these characters are excluded from calculation of the consistency index. One of the ten trees is depicted in Fig. 2. Relationships among supertribe *Triticodae* and tribes *Meliceae, Stipeae, Poeae,* and *Aveneae* do not vary among these cladograms, nor do generic compositions of the five groups; the cladograms vary only in relationships among genera within the five groups. Within *Meliceae*, the two species of *Glyceria* vary in position relative to *Melica*. Within *Poeae,* three subgroups consistently show the same internal structure as in Fig. 2 (*Catabrosa/Puccinellia/Sclerochloa/Sesleria*;

Arctagrostis/Poa; and *Festuca/Leucopoa*), although their relationships to one another and to *Bellardiochloa* and *Dactylis* vary.

Alternative arrangements of the tribes result in cladograms that are several steps longer. If the positions of *Meliceae* and *Stipeae* are exchanged, as is suggested by some morphological and anatomical data (MACFARLANE & WATSON 1982, BARKWORTH & EVERETT 1987), the resulting tree is 3 steps (2.3%) longer. If, instead, *Meliceae* are placed between *Triticodae* and *Poeae/Aveneae*, the tree is 5 steps (3.8%) longer.

When Dollo parsimony is employed (prohibiting parallel site gains), the generic composition of the tribes remains stable. However, the resulting trees are at least 17 steps longer (0.55 consistency index), the *Poeae* are rearranged (with *Poa* becoming sister group to the rest of the tribe), and a tree in which *Stipeae* diverge from the rest of the *Pooideae* before *Meliceae* is one step (0.69%) shorter than one in which *Meliceae* diverge before *Stipeae*. Further discussion of phylogenetic structure below refers to results under global parsimony.

Five monophyletic groups ("types") thus are identified within *Pooideae*. We designate the chloroplast genomes occurring in these clades the *Meliceae* type, *Stipeae* type, *Triticodae* type, *Aveneae* type, and *Poeae* type. These chloroplast genome types are cladistically defined entities; because reversals occur, a species within a particular chloroplast genome type may not exhibit all of its synapomorphies, except in a historical sense. The *Meliceae* chloroplast genome type occurs in *Glyceria, Melica*, and *Schizachne* (Fig. 2 and Table 2). The *Stipeae* type occurs in *Piptatherum* and *Stipa*. The *Triticodae* type occurs in *Brachypodium* and *Bromus*; maps of *Hordeum* (POULSEN 1983) and *Triticum* (BOWMAN & al. 1981) are consistent with this genome type, as are single digest RFLP patterns of *Hordeum, Secale*, and *Triticum* (ENOMOTO & al. 1985); when the latter two genera were entered into the analysis they formed a clade with *Hordeum*.

The *Aveneae* chloroplast genome type occurs in *Arrhenatherum, Briza, Chascolytrum, Deschampsia, Microbriza*, and *Torreyochloa* [*Briza* sensu lato, including *Chasolytrum* and *Microbriza*, may be polyphyletic on the basis of RS (SORENG & DAVIS, unpubl.) and other data (MATTHEI 1975)]. Available single digest patterns of *Avena* (ENOMOTO & al. 1985) are consistent with maps of *Aveneae* chloroplast genomes.

Finally, the *Poeae* chloroplast genome type occurs in *Arctagrostis, Bellardiochloa, Catabrosa, Dactylis, Festuca, Leucopoa, Poa, Puccinellia, Sclerochloa*, and *Sesleria*. Digest patterns of *Lolium multiflorum* (LEHVÄSHLAIHO & al. 1987) are consistent with maps of *Festuca* chloroplast genomes.

Discussion

Our analysis of restriction site variation differs from previous systematic studies of grasses based on molecular data in the large number of genera and tribes sampled, the employment of cladistic analysis, and the discovery of a substantial degree of homoplasy. The degree of character consistency in our analysis is lower than has been reported in studies confined to one or a few closely related genera (PALMER & al. 1988), but comparable to those involving larger numbers of genera (PALMER & al. 1988, JANSEN & PALMER 1988).

Our data support the inclusion of *Stipeae* and *Meliceae* within *Pooideae*, and

suggest that among these tribes *Meliceae* – not *Stipeae* – were the first to diverge. Although it has been argued that *Stipeae* should be excluded from *Pooideae* (MACFARLANE & WATSON 1982, BARKWORTH & EVERETT 1987), the morphological characters by which *Stipeae* are distinguished apparently are plesiomorphic in *Pooideae* (e.g., dome-shaped subsidiary cells; E. A. KELLOGG, unpubl.), autapomorphic for *Stipeae* (e.g., the pointed callus), or in other instances occur sporadically elsewhere in *Pooideae* (e.g., disarticulating leaf blades, three lodicules).

If Fig. 2 represents actual generic relationships, it appears to be most parsimonious to view the generalized pooid spikelet type (multiple florets, glumes shorter than the proximal lemma, and lemmas unawned or awned apically or subapically from a bifid apex) as plesiomorphic in *Pooideae*. This spikelet type occurs in *Meliceae, Triticodae,* and *Poeae,* as well as in *Briza, Chascolytrum,* and *Torreyochloa* of the *Aveneae*. Departures from this morphology occur as reduction to one floret per spikelet (in *Stipeae, Aveneae, Triticeae,* and *Poeae*, e.g., *Arctagrostis*); insertion of awns on the back of the lemma (*Aveneae*); and presence of glumes longer than the proximal lemmas (*Stipeae* and *Aveneae*).

If the character of glumes shorter than the lemmas is plesiomorphic within *Pooideae*, or at least within the smallest lineage that includes *Triticodae, Aveneae,* and *Poeae*, there is no incongruity in the co-occurrence of the *Aveneae* chloroplast genome type with the pooid spikelet type in *Briza, Chascolytrum*, and *Torreyochloa.* We interpret a broadly defined *Aveneae*, including these three genera, as plesiomorphically pooid in spikelet morphology. Monophylesis of the *Aveneae* spikelet type (with long glumes, and lemmas dorsally awned) within *Aveneae* remains a question, for *Arrhenatherum* and *Deschampsia* (which exhibit this form of spikelet) do not constitute a monophyletic group in Fig. 2. However, at this stage of our inquiry we feel that cladistic structure within tribes remains quite tentative, and we defer this question until more data are available.

Though we do not assert that *Danthonia* is sister taxon of the *Pooideae*, we acknowledge the long-recognized resemblance of spikelets of *Danthonia* (and related genera of *Arundinoideae*) to those of many genera of *Aveneae,* particularly in the character of glumes longer than the lemmas. Our employment of *Danthonia* as outgroup to the *Pooideae* therefore raises the question of whether long glumes are plesiomorphic in *Pooideae*. If actual cladistic relationships are as depicted in Fig. 2, and if long glumes are plesiomorphic in *Pooideae*, and if the occurrence of this character in *Aveneae* represents a retained plesiomorphy, rather than a reversal, then short glumes have evolved independently in *Meliceae, Triticodae, Poeae*, and *Aveneae* (i.e., *Briza, Chascolytrum*, and *Torreyochloa*). Clearly, the more parsimonious conclusion, given the structure we detect, is that short glumes are plesiomorphic in *Pooideae*.

Karyotype evolution in the *Pooideae* has been the subject of much debate (STEBBINS 1956, SHARMA 1979). One widely discussed trend is from the small or medium size chromosomes found in most nonpooid grasses, as well as in *Meliceae, Stipeae*, and some *Triticodae* (*Brachypodium* and *Bromus* sect. *Ceratochloa*), to the large chromosomes of *Poeae, Aveneae*, and other *Triticodae*. The cladistic structure we detect suggests two alternative phylogenies for these characters. One possibility is that large chromosomes evolved at least twice, once in the *Triticodae*, and again in the *Aveneae/Poeae* lineage. The second possibility is that large chromosomes evolved once, after divergence of *Stipeae* from the *Triticodae/Aveneae/Poeae* lineage,

and that the occurrence of small chromosomes in *Brachypodium* and some species of *Bromus* derives from a reversal within *Triticodae*. Both of these histories include the possibility that *Bromus*, which is polymorphic for chromosome size, is paraphyletic.

Another hypothesized transformation sequence involves derivation of the base chromosome number x = 7 (found in the majority of *Triticodae, Poeae,* and *Aveneae*) by reduction from higher numbers, such as x = 10, 11, and 12, as found in other grass subfamilies and in *Meliceae* and *Stipeae* (STEBBINS 1956). This sequence is consistent with the cladistic structure we detect within *Pooideae*. In *Meliceae,* the numbers x = 10, 9, and rarely 8 are found, and in *Stipeae,* x = 8, 10, 11, and 12 [*Achnatherum* (*Stipeae*) also may have x = 7 (TSVELEV 1983)]. Within *Triticodae,* x = 7 and 9 occur in *Brachypodium* (x = 5 in one section of annuals), but only x = 7 is found in *Bromeae* and *Triticeae*. Although there is variation from x = 7 in both *Poeae* and *Aveneae*, higher numbers are rare (restricted to derived annuals), and lower numbers might be interpreted as having been derived via descending aneuploidy within genera (*Anthoxanthum, Briza, Holcus, Milium, Phalaris, Phleum*, etc.), or as descending aneuploidy among related genera (*Catabrosa, Catabrosella, Colpodium*; TSVELEV 1983, CLAYTON & RENVOIZE 1986). Thus, it is possible that x = 7 became established before the divergence of *Triticodae* and *Poeae/Aveneae*.

An alternative interpretation of chromosome numbers suggests that x = 6 is plesiomorphic in *Poaceae* and x = 7 in *Pooideae* (excluding *Stipeae*; SHARMA 1979). Reports of x = 6 in a few genera of *Arundinoideae*, including *Danthonia* (DE WET 1954), are consistent with this hypothesis. If true, the cladistic structure we detect would necessitate a direct transition from x = 6 to x = 7 to account for the presence of the latter number in *Pooideae*, and also would appear to require independent transitions to x = 8 and higher numbers in *Meliceae* and *Stipeae*.

Lodicule types also are significant in assessment of relationships of *Meliceae* (JIRASEK & JOZIFOVA 1968, JIRASEK 1969). The truncate/nonvascularized lodicules of *Meliceae* may be transitional between the truncate/vascularized lodicules that occur in *Chloridoideae, Panicoideae*, and most *Arundinoideae* (STEBBINS 1956), on the one hand, and the lanceolate/nonvascularized lodicules of other *Pooideae*. A transition series from truncate/vascularized to truncate/nonvascularized to lanceolate/nonvascularized is consistent with *Meliceae* being sister group to the rest of *Pooideae*, though it does not account for the relationship between lodicules of *Pooideae* and the lanceolate/vascularized lodicules of *Bambusoideae*.

Chloroplast DNA RS patterns are consistent with present tribal alliances of most of the genera sampled, but in several cases the phylogenetic analysis suggests a need for re-evaluation of affinities. *Catabrosa*, often placed in *Meliceae*, shares a series of RS apomorphies with *Puccinellia* and *Sclerochloa* of the *Poeae*. *Briza, Chascolytrum, Microbriza,* and *Torreyochloa* are included in *Poeae* in most current taxonomic treatments, but have the *Aveneae* chloroplast genome type [*Torreyochloa* was distinguished only recently from *Glyceria* (*Meliceae*) (CHURCH 1949), and now is often submerged within *Puccinellia*]. The placement of *Arctagrostis* [traditionally included in *Aveneae* (*Agrostideae*)] near *Poa* (TSVELEV 1983, CLAYTON & RENVOIZE 1986) is supported by our analysis, and as mentioned above, may represent a case, apparently common in the grasses, of parallel reduction to a single-flowered spikelet.

The sharp distinction between chloroplast genome types of *Aveneae* and *Poeae* contrasts with the current absence of a well-defined morphological boundary be-

tween these tribes. The discovery of *Aveneae* chloroplast genome types in *Briza, Chascolytrum, Microbriza*, and *Torreyochloa*, genera usually unquestioned as *Poeae*, was a complete surprise. The parallel development of a series of restriction site gains is an extremely unlikely event (PALMER 1987). Alternative explanations for the observed character distributions include divergence from an ancestor polymorphic for the *Aveneae* and *Poeae* chloroplast genome types; multiple transfers of chloroplast genomes from the traditional *Aveneae* to the traditional *Poeae* (subsequent to divergence of the two chloroplast genome types); diversification of these four genera from a common pooid ancestor following a single chloroplast genome transfer from *Aveneae* to that ancestor. Although we cannot rule out intertribal transfer of chloroplast genomes, even a single such event seems highly improbable. No cases are known of hybridization between the *Poeae* and *Aveneae*, as delimited by our analysis. Furthermore, we emphasize that there is no inconsistency between the co-occurrence of the *Aveneae* chloroplast genome type and the pooid spikelet type, if the divergence of the *Aveneae* and *Poeae* chloroplast genomes preceded the origin of the avenoid spikelet. This leads us to suggest again that the pooid spikelet type is plesiomorphic in the *Poeae/Aveneae* clade, and that it is retained in *Briza, Chascolytrum, Microbriza,* and *Torreyochloa.*

We conclude that further investigation of chloroplast DNA RS phylogenies using cladistic analysis, in combination with critical re-evaluation and phylogenetic analysis of traditional characters, will yield an improved resolution of generic affinities and higher order phylogenetic structure within the *Poaceae.*

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References

BARKWORTH, M. E., EVERETT, J., 1987: Evolution in the *Stipeae*: identification and relationships of its monophyletic taxa. – In SODERSTROM, T. R., HILU, K. W., CAMPBELL, C. S., BARKWORTH, M. E., (Eds.): Grass systematics and evolution, pp. 251–264. – Washington: Smithsonian Institution.

BAUM, B. R., 1987: Numerical taxonomic analyses of the *Poaceae*. – In SODERSTROM, T. R., HILU, K. W., CAMPBELL, C. S., BARKWORTH, M. E., (Eds.): Grass systematics and evolution, pp. 334–342. – Washington: Smithsonian Institution.

BOWMAN, C. M., KOLLER, B., DELIUS, H., DYER, T. A., 1981: A physical map of wheat chloroplast DNA showing the location of the structural genes for the ribosomal RNAs and the large subunit of ribulose 1,5-bisphosphate carboxylase. – Mol. Gen. Genet. **183**: 93–101.

CHURCH, G. L., 1949: A cytotaxonomic study of *Glyceria* and *Puccinellia*. – Amer. J. Bot. **36**: 155–165.

– 1952: The genus *Torreyochloa*. – Rhodora **54**: 197–200.

CLAUSEN, R. T., 1952: Suggestion for the assignment of *Torreyochloa* to *Puccinellia*. – Rhodora **54**: 42–45.

CLAYTON, W. D., RENVOIZE, S. A., 1986: Genera graminum. – Kew Bull. Add. Ser. **13**: 1–389.

DALLY, A. M., SECOND, G., 1989: Chloroplast DNA isolation from higher plants: an improved non-aqueous method. – Pl. Mol. Biol. Reporter **7**: 135–143.

DANG, L. H., PRING, D. R., 1986: A physical map of the *Sorghum* chloroplast genome. – Pl. Mol. Biol. **6**: 119–123.

DE WET, J. M. J., 1954: The genus *Danthonia* in grass phylogeny. – Amer. J. Bot. **41**: 204–211.

DEBRY, R. W., SLADE, N. A., 1985: Cladistic analysis of restriction endonuclease cleavage maps within a maximum-likelihood framework. – Syst. Zool. **34**: 21–34.

ENOMOTO, S.-I., OGIHARA, Y., TSUNEWAKI, K., 1985: Studies on the original crop species by restriction endonuclease analysis of organellar DNA 1. Phylogenetic relationships among ten cereals revealed by the restriction fragment patterns of chloroplast DNA. – Japan. J. Genet. **60**: 411–424.

FARRIS, J. S., 1988: HENNIG 86. ver. 1.5 [computer software and manual] (publ. by author).

FEINBERG, A. P., VOGELSTEIN, B., 1984: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. – Anal. Biochem. **137**: 266–267.

FELSENSTEIN, J., 1987: PHYLIP. ver. 3.0 [computer software and manual] (publ. by author).

HAMBY, R. K., ZIMMER, E. A., 1988: Ribosomal RNA sequences for inferring phylogeny within the grass family (*Poaceae*). – Pl. Syst. Evol. **160**: 29–37.

HILU, K. W., WRIGHT, K., 1982: Systematics of *Gramineae:* a cluster analysis study. – Taxon **31**: 9–36.

HIRAI, A., ISHIBASHI, T., MORIKAMI, A., IWATSUKI, N., SHINOZOKI, K., SUGIURA, M., 1985: Rice chloroplast DNA: a physical map and the location of the genes for the large subunit of ribulose 1,5-bisphosphate carboxylase and the 32 KD photosystem II reaction center protein. – Theor. Appl. Genet. **70**: 117–122.

HIRATSUKA, J., SHIMADA, H., WHITTIER, R., ISHIBASHI, T., SAKANOTO, M., MORI, M., KONDO, C., HONJI, Y., SUN, S.-R., MENG, B.-Y., LI, Y.-Q., KANNO, A., NISHIZAWA, Y., HIRAI, A., SHINOZAKI, K., SUGIURA, M., 1989: The complete sequence of the rice (*Oryza sativa*) chloroplast genome: Intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. – Mol. Gen. Genet. **217**: 185–194.

HOWE, C. J., BARKER, R. F., BOWMAN, C. M., DYER, T. A., 1988: Common features of three inversions in wheat chloroplast DNA. – Curr. Genet. **13**: 343–349.

JANSEN, R. K., PALMER, J. D., 1988: Phylogenetic implications of chloroplast DNA restriction site variation in the *Mutisieae* (*Asteraceae*). – Amer. J. Bot. **75**: 753–766.

JIRASEK, V., 1969: Morphologie der Schüppchen (Lodiculae) von Gräsern und ihre Terminologie. Ein weiterer Beitrag zur Kenntnis des Baues der Lodiculae. – Acta Univ. Carolinea Biologica **1968**: 321–344.

– JOZIFOVA, M., 1968: Morphology of locidules, their variability and importance in the taxonomy of the *Poaceae* family. – Bol. Soc. Argentina Bot. **12**: 324–349.

KELLOGG, E. A., CAMPBELL, C. S., 1987: Phylogenetic analyses of the *Gramineae*. – In SODERSTROM, T. R., HILU, K. W., CAMPBELL, C. S., BARKWORTH, M. E., (Eds.): Grass systematics and evolution, pp. 310–322. – Washington: Smithsonian Institution.

LARRINUA, I. M., MUSKAVITCH, K. M. T., GUBBINS, E. J., BOGORAD, L., 1983: A detailed restriction endonuclease site map of the *Zea mays* plastid genome. – Pl. Mol. Biol. **2**: 129–140.

LEHVÄSHLAIHO, H., SAURA, A., LOKKI, J., 1987: Chloroplast DNA variation in the grass tribe *Festuceae*. – Theor. Appl. Genet. **74**: 298–302.

MACFARLANE, T. D., 1987: *Poaceae* subfamily *Pooideae*. – In SODERSTROM, T. R., HILU, K. W., CAMPBELL, C. S., BARKWORTH, M. E., (Eds.): Grass systematics and evolution, pp. 265–276. – Washington: Smithsonian Institution.

– WATSON, L., 1980: The circumscription of *Poaceae* subfamily *Pooideae* with notes on some controversial genera. – Taxon **29**: 645–666.

– – 1982: The classification of *Poaceae* subfamily *Pooideae*. – Taxon **31**: 178–203.

MANIATIS, T., FRITSCH, E. F., SAMBROCK, J., 1982: Molecular cloning. A laboratory manual. – Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

MATTHEI, O., 1975: Der *Briza*-Komplex in Südamerika: *Briza, Calotheca, Chascolytrum, Poidium* (*Gramineae*). – Willdenowia **8**: 1–168.

MOON, E., WU, R., 1988: Organization and nucleotide sequence of genes of both junctions between the two inverted repeats and the large single-copy region in the rice chloroplast genome. – Gene **70**: 1–12.

PALMER, J. D., 1983: Chloroplast DNA exists in two orientations. – Nature **301**: 92–93.

– 1986: Isolation and structural analysis of chloroplast DNA. – Methods Enzymol. **118**: 167–186.

– 1987: Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. – Amer. Naturalist **30**: S 6–S 29.

– JANSEN, R. K., MICHAELS, H. J., CHASE, M. W., MANHART, J. R., 1988: Chloroplast DNA variation and plant phylogeny. – Annu. Missouri Bot. Gard. **75**: 1180–1206.

POULSEN, C., 1983: The barley chloroplast genome: physical structure and transcriptional activity in vivo. – Carlsberg Res. Commun. **48**: 57–80.

QUIGLEY, F., WEIL, J. H., 1985: Organization and sequence of five tRNA genes and of an unidentified reading frame in the wheat chloroplast genome: evidence for gene rearrangements during the evolution of chloroplast genomes. – Curr. Genet. **9**: 495–503.

SHARMA, M. L., 1979: Some considerations on the phylogeny and chromosomal evolution in grasses. – Cytologia **44**: 679–685.

SODERSTROM, T. R., HILU, K. W., CAMPBELL, C. S., BARKWORTH, M. E., (Eds.), 1987: Grass systematics and evolution. – Washington: Smithsonian Institution.

SØGAARD, B., VON WETTSTEIN-KNOWLES, P., 1987: Barley: genes and chromosomes. – Carlsberg Res. Commun. **52**: 123–196.

SORENG, R. J., 1990: Chloroplast DNA phylogenetics and biogeography in a reticulating group: a study in *Poa* L. (*Poaceae*). – Amer. J. Bot. (in press).

SOUTHERN, E. M., 1975: Detection of specific sequences among DNA fragments separated by gel electrophoresis. – J. Mol. Biol. **98**: 503–517.

STEBBINS, G. L., 1956: Cytogenetics and evolution of the grass family. – Amer. J. Bot. **43**: 890–905.

– CRAMPTON, B., 1961: A suggested revision of the grass genera of temperate North America. – Rec. Adv. Bot. **1**: 133–145.

TATEOKA, T., 1957: Miscellaneous papers on the phylogeny of *Poaceae*. 10. Proposition. – J. Japan Bot. **32**: 275–287.

TEMPLETON, A. R., 1983: Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. – Evolution **37**: 221–244.

THOMAS, K., WOOD, B. J., BASSETT, C. L., RAWSON, J. R. Y., 1984: A restriction endonuclease map of the chloroplast genome of pearl millet. – Curr. Genet. **8**: 291–297.

TSVELEV, N. N., 1983: Zlaki SSSR [English translation of 1976 Russian edn.]. – New Delhi: for Smithsonian Inst. by Amerind Publ. Co.

VEDEL, F., LEBACQ, P., QUETIER, F., 1980: Cytoplasmic DNA variation and relationships in cereal genomes. – Theor. Appl. Genet. **58**: 219–224.

WATSON, L., CLIFFORD, H. T., DALLWITZ, M. J., 1985: The classification of *Poaceae:* subfamilies and supertribes. – Austral. J. Bot. **33**: 433–484.

ZURAWSKI, G., CLEGG, M. T., 1987: Evolution of higher plant chloroplast DNA-encoded genes: implications for structure-function and phylogenetic studies. – Annu. Rev. Pl. Physiol. **38**: 391–418.

– – BROWN, A. H. D., 1984: The nature of nucleotide sequence divergence between barley and maize chloroplast DNA. – Genetics **106**: 735–749.

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