Theor Appl Genet (1995) 90:389–398

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Molecular phylogeny of the Pooideae (Poaceae) based on nuclear rDNA (ITS) sequences

Received: 21 July 1994 / Accepted: 28 July 1994

Abstract Phylogenetic relationships of the Poaceae subfamily, Pooideae, were estimated from the sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The entire ITS region of 25 species belonging to 19 genera representing seven tribes was directly sequenced from polymerase chain reaction (PCR)-amplified DNA fragments. The published sequence of rice, *Oryza sativa*, was used as the outgroup. Sequences of these taxa were analyzed with maximum parsimony (PAUP) and the neighbor-joining distance method (NJ). Among the tribes, the Stipeae, Meliceae and Brachypodieae, all with small chromosomes and a basic number more than $x = 7$, diverged in succession. The Poeae, Aveneae, Bromeae and Triticeae, with large chromosomes and a basic number of $x = 7$, form a monophyletic clade. The Poeae and Aveneae are the sister group of the Bromeae and Triticeae. On the ITS tree, the Brachypodieae is distantly related to the Triticeae and Bromeae, which differs from the phylogenies based on restriction-site variation of cpDNA and morphological characters. The phylogenetic relationships of the seven pooid tribes inferred from the ITS sequences are highly concordant with the cytogenetic evidence that the reduction in chromosome number and the increase in chromosome size evolved only once in the pooids and pre-dated the divergence of the Poeae, Aveneae, Bromeae and Triticeae.

Key words Poaceae · Pooideae · rDNA sequences Molecular phylogeny

This paper is a cooperative investigation of USDA-ARS and the Utah Agricultural Experiment Station. Logan, Utah 84322. Journal Paper No. 4581

Communicated by Dooner

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Introduction

The Poaceae subfamily, the Pooideae, has been divided into from four to 27 tribes depending on how the boundary of the subfamily was defined (Tateoka 1957; Stebbins and Crampton 1961; Macfarlane and Watson 1982; Gould and Shaw 1983; Watson et al. 1985; Clayton and Renvoize 1986; Macfarlane 1987; Tzvelev 1989). Macfarlane (1987) divided the Pooideae into two supertribes: the Poodae, including the Poeae, Seslerieae, Aveneae and Meliceae, and the Triticodae, including the Brachypodieae, Bromeae and Triticeae. Macfarlane excluded the Stipeae from the subfamily, but other authors have generally included it. Phylogenetic relationships among the tribes based on analyses of morphological data, either by phenetic or cladistic methods, have failed to define a clear evolutionary pattern (Macfarlane and Watson 1980, 1982; Hilu and Wright 1982; Watson et al. 1985; Baum 1987; Kellogg and Campbell 1987; Kellogg and Watson 1993).

Morphological characters have traditionally been the key criteria in making taxonomic decisions. Anatomical and micromorphological features are very useful, but data for these characters are too scattered and incomplete across the taxa to be of value in delimiting taxonomic categories. Early chromosome studies by Avdulov (1931), Kihara (1954), Stebbins (1956), and the more recent ones by Dewey (1982, 1984), have provided important information on genome differentiation, the origin of polyploids, and the reticulate evolution of the Poaceae. However, chromosome morphology, size, and numbers are not discrete characters for taxonomic delimitation but rather an indication of trends of evolutionary direction. Cytogenetic information reflects genetic relatedness whereas morphological characters represent functional information and can sometimes be

phylogenetically misleading due to parallel evolution and rapid adaptive radiation.

Physiological traits, such as the nature of the starch grain (Tateoka 1962), the storage of fructosans (Smith 1973), and serological data (Watson and Knox 1976), are incomplete in the taxa examined and can also be influenced by adaptive radiation.

In an early molecular study of repeated DNA sequences, Flavell (1982) concluded that *Aegilops* species are more closely related to *Triticum monococcum* than to *Secale*, *Hordeum* or *Avena*. Using restriction-site variation of cpDNA, Enomoto et al. (1985) showed that *Triticum* and *Secale* are closer to *Hordeum* than to *Avena*. Recently, Soreng et al. (1990) reported a more comprehensive molecular phylogeny of the Pooideae based on the restriction-site variation of cpDNA. They studied seven tribes, the Meliceae, Stipeae, Poeae, Aveneae, Brachypodieae, Bromeae and Triticeae, and concluded that the Pooideae is monophyletic and supported the inclusion of the Meliceae and Stipeae in the subfamily.

Restriction-site variations or sequences of the chloroplast genome are powerful tools for studying the phylogenetic relationships of plants (Enomoto et al. 1985; Zurawski and Clegg 1987; Soreng et al.1990; Kellogg 1992). However, because the chloroplast genome is cytoplasmic, it is inherited unidirectionally through maternal lines. The rates of nucleotide substitution in plant chloroplast and mitochondrial genomes are much slower than that of nuclear genes (Wolfe et al. 1987). The divergence distance estimated from the chloroplast genome may not directly reflect that of the species. Phylogeny inferred from the cytoplasmic genome may also obscure the true evolutionary direction of closely related species because interspecific hybridization among plants often occurs repeatedly in both directions. On the other hand, because of sexual segregation and recombination, the nuclear genome could also be phylogenetically misleading.

Our previous study on the assessment of the internal transcribed spacer (ITS) region of the rDNA of the nuclear genome showed promising results for the phylogenetic study of grasses (Hsiao et al. 1994). ITS sequence data have also been used successfully to reconstruct the phylogeny of the Compositae by Baldwin (1992, 1993), of the Winteraceae by Suh et al. (1993), of the Fabaceae by Wojciechowski et al. (1993), and of the Ranunculaceae by S. Hodges (personal communication). In the present study we have sequenced the entire ITS region of 25 pooid taxa for direct comparison with phylogenies based on data from nuclear and chloroplast genomes. Comparisons between phylogenies inferred from both genomes would provide a better basis for assessing the true species relationships.

Materials and methods

Plant samples

The names, authorities, source, and GenBank accession numbers of the 25 pooid species selected for this study as well as that of the outgroup *Oryza sativa* are listed in Table 1. Total DNA was extracted from fresh leaves as described in our previous report (Hsiao et al. 1994) except that the DNA samples of *Glyceria striata*, *G. borealis* and *Briza minor* were extracted from herbarium specimens. Briefly, fresh or dry leaves, leaves stored at −80 °C or preserved in absolute ethanol (at −20 °C), were extracted with phenol/chloroform. Some DNA samples were extracted by the CTAB method (Lassner et al. 1989; Williams et al. 1993). The two DNA extraction methods were equally good for the polymerase chain reaction (PCR).

PCR amplification and sequencing strategy

The PCR amplification and dideoxy sequencing protocol were described previously (Hsiao et al. 1994). The entire ITS region was amplified with primers ITSL (5′-TCGTAACAAGGTTTCCGTAGGTG-3′) and ITS4 (5′-TCCTCCGCTTATTGATA TGC-3′), and was directly sequenced from the double- stranded DNA fragment with one of the amplification primers or one of the internal sequencing primers, ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) or ITS3 (5′-GCATCGATGAAGAACGCAGC-3′). During the latter half of the study, the PCR products of some species were sequenced on an ABI 373A autosequencer (Applied Biosystems) using *Taq* polymerase and dye-terminators according to the ABI autosequencing protocol.

Sequence analysis

Sequences of the entire ITS region of 25 pooid species together with that of *O. sativa* (Takaiwa et al. 1985) were aligned with the LINEUP program of the University of Wisconsin Genetics Computer Group (UWGCG) and the CLUSTAL V multiple sequence alignment programs (Higgins et al. 1992). Phylogenetic relationships were analyzed by unweighted maximum parsimony (PAUP, version 3.1.1. Swofford 1993) using the "heuristics" search option with the "closest" addition sequence and TBR (tree bisection-reconnection) swapping. Gaps were treated as missing data.

The same aligned sequences were also analyzed by the neighbor-joining (NJ) distance method (MEGA, Molecular Evolutionary Genetics Analysis, version 1.0, Kumar et al. 1993) using the Kimura two-parameter distance and the pairwise gap deletion option.

Results

Sequence variation

The PCR-amplified DNA fragments of most species showed a clean single band product when examined on an agarose gel. DNA fragments amplified from *Festuca arundinacea* gave an ambiguous sequence, possibly due to an endophyte infection. Subsequently, an endophyte-free species, *F. mairei*, was used. Known recent hybrids also gave ambiguous sequences, possibly due to the presence of two parental sequences.

The lengths of the entire ITS sequences of the 25 pooid species analyzed varied from 585 to 602 bp. The ITS 1 region ranged from 214 to 221 bp, and the ITS 2 region ranged from 205 to 221 bp. The length of the 5.8*s* subunit was uniformly 164 bp in all species. Aligned sequences of the 25 taxa and the outgroup, *O. sativa*, are shown in Fig. 1.

The pairwise nucleotide-sequence divergence (Kimura's two-parameter distance) among the pooid species (Table 2) ranged from 1.18% between *Bromus diandrus* and *B. tectorum* to 29.20% between *G. borealis* and *B. briziformis*. There was no sequence variation

Table 1 Twenty-five species of Pooideae and one species of *Oryzeae* included in the ITS sequence analysis

Subfamily, tribe, species	Abbreviation	Source[a]	GenBank accession No.
Bambusoideae			
Oryzeae			
Oryza sativa L.	ORSA	Takaiwa et al. 1985	—
Pooideae			
Aveneae			
Avena longiglumis Durieu	AVLO	S. Fritz, CN	Z11758
Briza minor L.[b]	BRMI	UTC 85068	L36510
Deschampsia cespitosa Beauv.	DECE	T-19731	L36513
Phalaris truncata auct. non Guss.	PHTR	N. J. Chatterton	L36522
Brachypodieae			
Brachypodium distachyon (L.) Beauv.	BRDI	R. Riggins, Calif.	L11578
Brachypodium sylvaticum (Huds.) Beauv.	BRSY	DJ 4075, USSR	L36511
Bromeae			
Bromus briziformis Fisch. & Mey.	BRBR	CH101	L36508
Bromus diandrus Roth	BRDI	CH 102	L36509
Bromus inermis Leysser	BRIN	CH 103	L11579
Bromus tectorum L.	BRTE	E. A. Kellogg	L36485
Meliceae			
Glyceria striata (Lam,) Hitchc.	GLST	MC 826	L36516
Glyceria borealis (Nash) Batchelder	GLBO	UTC 205678	L36515
Melica californica Scribner	MECA	MC 719	L36518
Melica imperfecta Trinius	MEIM	MC 715	L36519
Poeae			
Dactylis glomerata L.	DAGL	N. J. Chatterton	L36512
Festuca mairei St. -Yves (Endophyte free)	FEMA	C. West	L36514
Lolium perenne L.	LOPE	N. J. Chatterton	L36517
Stipeae			
Achnatherum hymenoides (Roemer & Schultes) Barkworth	ACHY	PI 478833	L36507
Nassella viridula (Trinius) Barkworth	NAVI	T. Jones, Londorm	L36521
Nassella leucotricha (Trinius & Ruprecht) Pohl.	NALE	M. Houck	L36520
Piptochaetium fimbriatum (Kunth) Hitchc.	PIFI	MB 5161	L36523
Triticeae			
Agropyron cristatum (L.) Gaertner	AGCR	PI 229574	L36480
Critesion californicum (Covas & Stebbins) Löve	CRCA	G. L. Stebbins	L36486
Psathyrostachys juncea (Fisch.) Nevski	PSJU	PI 206684	L36500
Thinopyrum bessarabicum (Savul. & Rayss) Löve	THBE	V. Jaaska	L36506

[a] UTC, Intermountain Herbarium, Utah; MB, M. E. Barkworth collection; MC, M. Curto collection; CH, C. Hsiao collection; DJ, D. A. Johnson collection; PI, USDA plant introduction station; T, part of USDA living collection of perennial Triticeae

[b] Tribal realignment suggested by Soreng et al. (1990)

between two individual plants of the same species, except for 3-bp differences between two individuals of *Achnatherum hymenoides* (Stipeae). However, we cannot rule out variation among accessions of the same species from distant geographical regions. Baldwin (1993) reported a 4.3% intraspecific divergence in *Calycadenia truncata* (Compositae), and there were two types of ITS sequences in clones of an individual of the Winteraceae (Suh et al. 1993). We did not detect such variation in pooid species in our PCR-based sequencing.

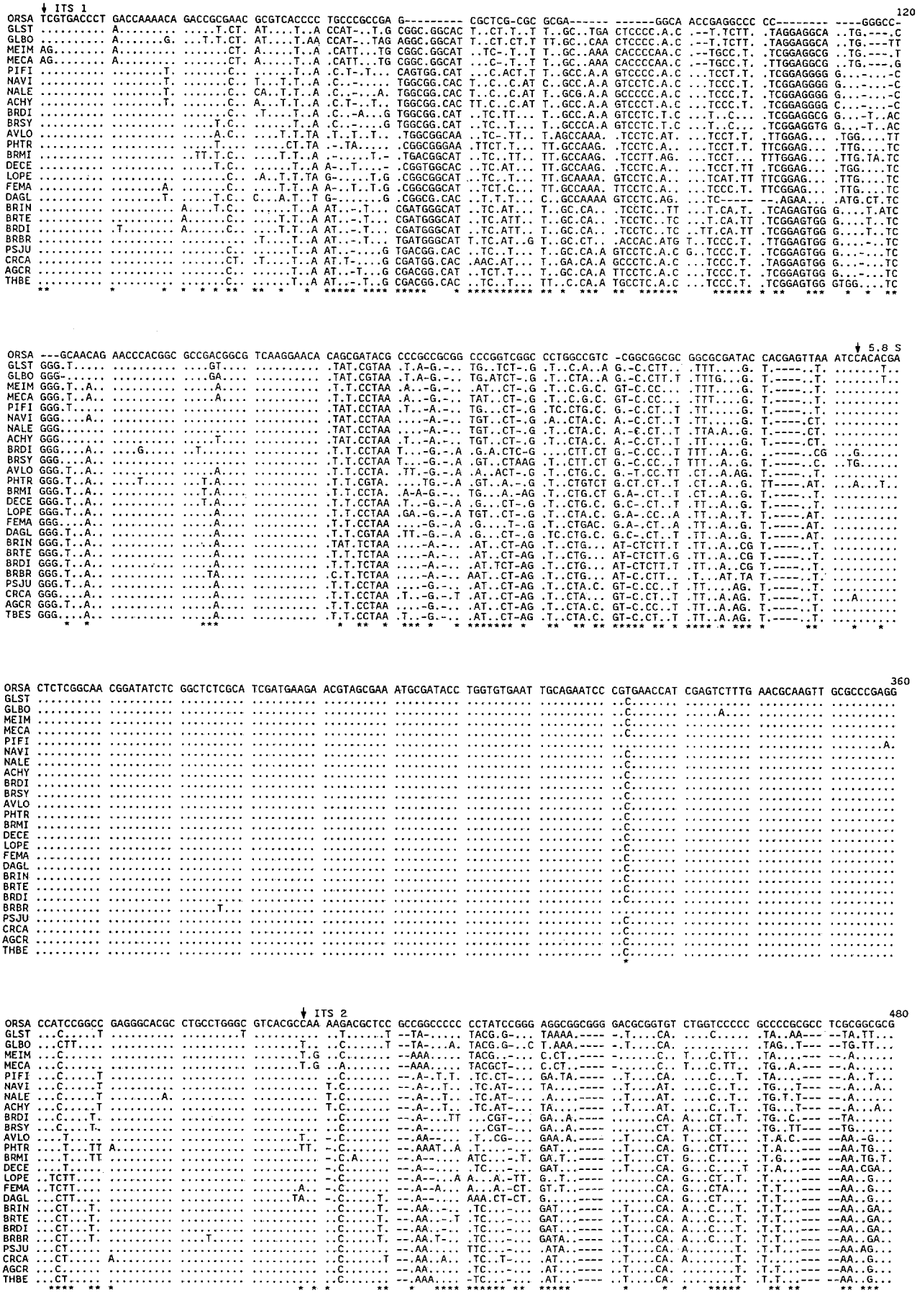
The alignment of the entire ITS sequences of 26 species (Fig. 1) resulted in 636 characters, of which 238 positions (37.4%) were potentially phylogenetically informative sites. The ITS 1 region had 115 informative sites in 233 positions (49.4%) and the ITS 2 region had 113 informative sites in 239 positions (47.3%). There were ten informative sites (6.1%) in the 5.8 *s* subunit region.

Gaps due to insertion/deletion events were introduced to align the sequences of the ITS 1 and ITS 2 regions. The largest gap (12 bp) was in the ITS 1 region of the *O. sativa* sequence. Gaps were correlated with particular species groups and were potentially phylogenetically informative. Baldwin (1993) also suggested that length mutation in the ITS region can be of potential value for phylogeny reconstruction.

The numbers of transitions/transversions of pairwise comparisons are listed in Table 2. Their ratios ranged from 0.75 between two *Bromus* species to 5.75 between two Stipeae species. The overall transition/transversion ratio of the subfamily, 1.93, was not strongly biased. It was not necessary to weight the transition and transversion for the phylogenetic analysis of tribes.

Phylogenetic inference

Maximum parsimony analysis of the aligned sequences (Fig. 1) yielded three maximally parsimonious trees. Each required 948 evolutionary steps (consistency index = 0.534, retention index = 0.690). The majority rule tree is shown in Fig. 2. Except for the boundary between the Aveneae and Poeae, the clades of each tribe


ITS 1
5.8 S
ITS 2
ORSA
GLST
GLBO
MEIM
MECA
PIFI
NAVI
NALE
ACHY
BRDI
BRSY
AVLO
PHTR
BRMI
DECE
LOPE
FEMA
DAGL
BRIN
BRTE
BRDI
BRBR
PSJU
CRCA
AGCR
THBE
TBES

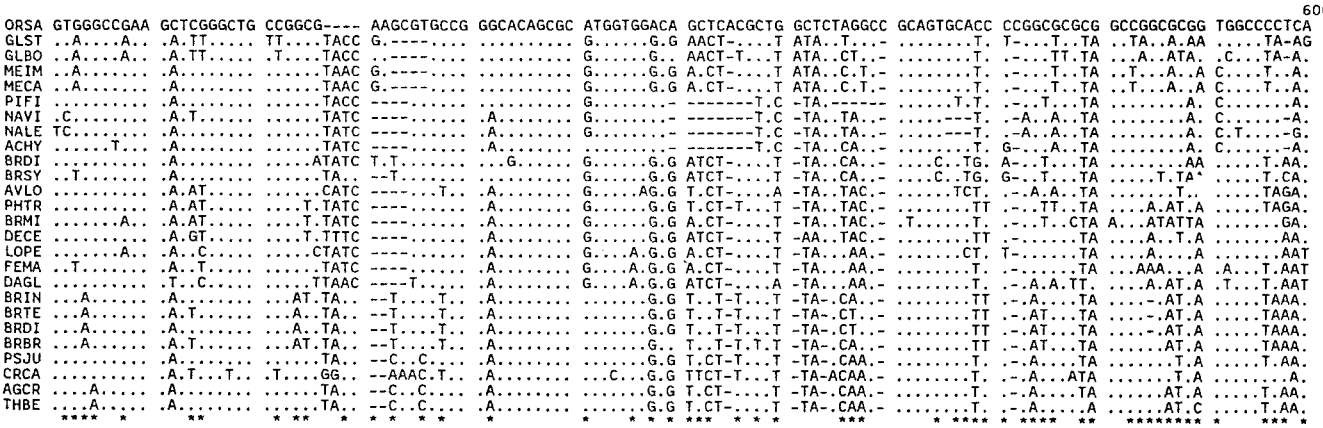


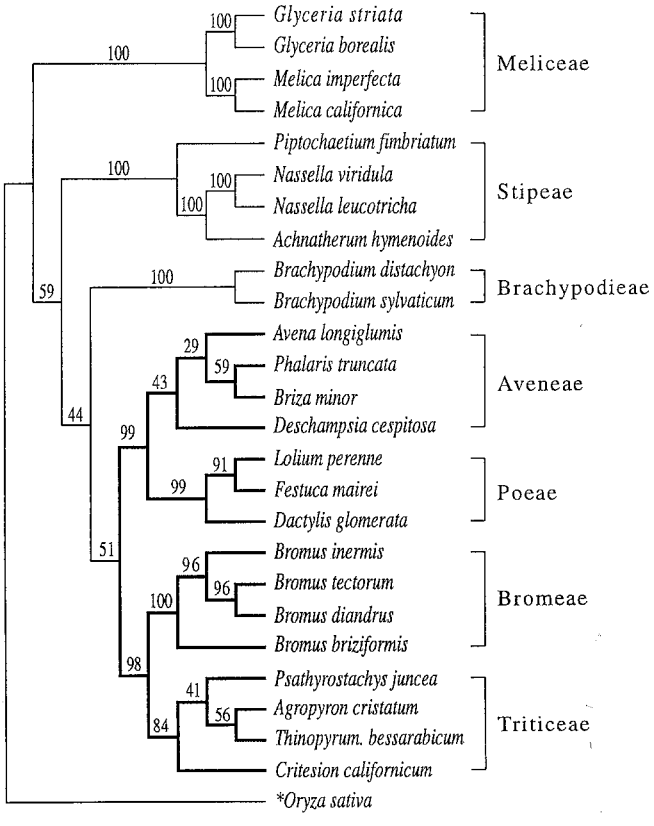
Fig. 1 Aligned sequence data of 25 pooid species and an outgroup, *O. sativa* (see Table 1 for abbrevation). *Numbers* indicate the consecutive positions of 1 to 636 (5′ to 3′) from the beginning of the ITS 1 region to the end of the ITS 2 region; *arrows* indicate the beginning of the ITS 1, 5.8*s* and ITS 2 regions; *dashes* denote gaps; *dots* denote identity to the *O. sativa* sequence; *asterisks* mark informative sites. The nucleotide-sequence data reported have been deposited with Genbank (see Table 1)

Table 2 Pairwise comparisons of nucleotide substitutions of 25 Pooideae species and an outgroup, *O. sativa*. The percent of sequence divergence using Kimura 2-parameter distance are shown above the diagonal. Direct counts of transitions/transversions are shown below the diagonal. See Table 1 for species abbreviations

	ORSA	GLST	GLBO	MEIM	MECA	PIFI	NAVI	NALE	ACHY	BRDI	BRSY	AVLO	PHTR
ORSA	–	31.13	34.80	25.53	26.36	21.68	22.47	23.53	23.67	25.62	25.19	27.69	29.22
GLST	83/55	–	9.29	14.72	14.48	18.96	20.20	21.40	19.61	23.59	21.97	23.90	24.62
GLBO	87/64	38/13	–	18.86	19.52	20.98	22.73	23.45	22.11	26.45	24.27	25.62	26.91
MEIM	64/55	52/26	64/33	–	1.53	16.10	17.37	17.99	17.48	19.42	17.51	20.01	21.49
MECA	68/54	49/28	65/35	7/2	–	16.06	17.09	17.72	17.20	19.85	17.94	20.45	23.17
PIFI	64/37	69/24	70/32	59/22	57/24	–	9.75	10.77	10.10	15.71	16.20	17.41	18.81
NAVI	59/46	69/30	71/39	60/27	57/29	44/8	–	3.33	4.06	16.78	15.24	18.72	20.35
NALE	63/46	72/32	72/41	59/31	56/33	48/9	12/7	–	5.53	18.34	16.77	20.60	22.26
ACHY	65/45	68/29	70/38	60/28	57/30	46/8	17/6	22/9	–	18.04	16.03	20.25	21.72
BRDI	66/55	75/41	78/50	59/40	59/42	60/21	63/23	67/26	68/24	–	5.23	20.40	21.13
BRSY	66/53	68/41	69/50	54/36	54/38	62/21	57/22	61/25	60/23	23/7	–	18.96	19.68
AVLO	73/54	74/42	77/46	59/42	59/44	62/25	64/29	69/32	69/31	63/40	55/42	–	12.59
PHTR	73/61	72/48	78/51	60/48	65/50	60/34	62/39	67/42	69/38	59/48	50/51	41/27	–
BRMI	73/57	68/48	72/52	67/42	68/44	60/31	65/34	70/37	69/35	65/44	54/43	51/24	40/30
DECE	63/52	64/41	72/45	55/37	60/39	49/22	49/27	56/30	55/28	50/38	42/41	43/17	34/22
LOPE	70/54	64/54	69/59	49/47	51/49	55/33	55/35	60/34	56/37	47/42	47/43	44/35	44/40
FEMA	67/55	65/52	64/55	44/47	48/49	55/32	53/35	57/38	58/36	49/45	45/44	42/34	36/40
DAGL	73/53	69/51	71/54	56/49	56/51	58/38	58/41	63/44	63/44	56/50	49/52	41/43	46/46
BRIN	81/50	87/36	88/43	72/30	77/32	70/18	70/23	76/25	73/25	74/31	66/33	61/32	57/36
BRTE	83/52	89/39	87/44	72/33	77/35	69/19	69/25	77/27	72/27	69/33	65/35	61/34	60/38
BRDI	83/52	90/37	88/42	71/33	76/35	68/19	70/25	76/27	71/27	70/33	64/35	60/34	61/38
BRBR	81/56	88/43	87/50	76/39	78/41	68/27	71/32	76/34	72/34	74/43	70/45	72/37	66/39
PSJU	65/50	70/38	70/45	47/32	52/34	63/14	59/19	64/21	62/21	59/29	51/31	49/28	52/34
CRCA	75/55	71/43	71/50	63/36	64/38	68/19	65/26	67/28	67/28	67/39	63/39	57/36	61/41
AGCR	64/50	69/37	72/44	46/29	51/31	62/13	60/18	63/20	64/20	53/32	48/34	51/26	49/35
THBE	63/51	72/40	70/47	54/30	59/32	64/15	62/20	64/22	64/22	61/34	53/36	54/27	52/36

	BRMI	DECE	LOPE	FEMA	DAGL	BRIN	BRTE	BRDI	BRBR	PSJU	CRCA	AGCR	THBE
ORSA	28.51	24.37	26.80	26.22	27.81	28.70	29.79	29.79	30.23	24.25	28.20	23.99	23.97
GLST	23.75	21.08	23.98	23.77	24.97	25.90	27.16	26.96	27.78	21.84	23.22	21.37	22.70
GLBO	25.72	23.98	26.44	24.21	26.21	27.84	27.80	27.60	29.20	23.42	24.83	23.70	23.78
MEIM	22.04	17.99	18.73	17.60	21.14	20.56	21.22	20.98	23.53	15.11	19.61	14.28	16.19
MECA	22.74	19.59	19.63	18.94	21.59	22.26	22.93	22.68	24.51	16.64	20.29	15.79	17.74
PIFI	18.51	13.75	17.41	17.20	19.48	17.64	17.62	17.38	19.09	15.05	17.24	14.62	15.51
NAVI	20.10	14.75	17.81	17.35	20.12	18.68	18.86	19.11	20.88	15.14	17.98	15.16	16.06
NALE	22.02	16.97	18.77	18.93	22.04	20.58	21.28	21.03	22.59	16.69	18.88	16.25	16.93
ACHY	21.18	16.22	18.37	18.62	21.90	19.72	19.91	19.66	21.45	16.14	18.76	16.39	16.83
BRDI	21.81	16.90	17.07	18.16	21.15	20.81	20.04	20.28	23.45	16.75	20.69	16.04	18.27
BRSY	18.98	15.76	17.28	17.05	19.94	19.25	19.44	19.21	22.81	15.34	19.67	15.30	16.83
AVLO	14.19	11.06	14.85	14.22	16.12	18.24	18.67	18.44	21.98	14.70	18.17	14.73	15.51
PHTR	12.93	10.14	15.72	14.04	17.70	18.10	19.22	19.46	20.86	16.54	20.11	16.08	16.86
BRMI	–	12.00	15.24	15.23	18.79	19.89	20.33	20.33	22.94	17.58	22.42	17.34	17.67
DECE	46/19	–	12.46	11.66	13.88	13.83	14.89	14.89	17.53	11.35	14.43	11.76	11.28
LOPE	52/29	37/31	–	7.23	12.62	19.83	19.59	19.79	22.65	14.61	19.19	14.80	15.57
FEMA	51/30	35/29	24/17	–	11.60	19.10	19.57	19.53	22.63	14.61	19.66	13.96	14.51
DAGL	60/36	37/37	37/31	32/31	–	19.63	21.29	21.04	24.21	16.74	20.34	16.27	17.05
BRIN	64/36	47/26	65/35	61/36	59/39	–	2.92	2.74	7.64	10.05	13.22	9.45	10.22
BRTE	64/38	50/28	64/35	63/36	64/41	13/4	–	1.18	7.63	11.04	13.83	10.42	11.21
BRDI	64/38	50/28	63/37	61/38	63/41	12/4	3/4	–	8.01	10.83	14.04	10.22	11.01
BRBR	71/42	59/31	72/40	71/41	71/46	27/16	25/18	27/18	–	12.83	16.32	13.22	13.86
PSJU	56/34	39/22	44/33	44/33	48/38	43/12	46/14	45/14	50/19	–	7.98	2.91	3.79
CRCA	68/43	46/30	56/42	58/42	56/46	50/21	51/23	52/23	56/30	29/16	–	8.55	8.17
AGCR	54/35	41/22	43/35	41/33	44/40	40/12	43/14	42/14	50/21	12/5	31/17	–	2.74
THBE	55/36	38/23	46/36	43/34	46/42	42/14	45/16	44/11	53/21	15/7	27/19	12/4	–

Fig. 2 Majority rule consensus of three maximonious parsimony trees. The *Asterisk* designates the outgroup species. *Numbers* on the branches are bootstrap values from 100 replicates of heuristic search analyses (PAUP). *Bold lines* indicate the pooid "core" clade



in the pooids were well defined and supported, as indicated by bootstrap values.

The same sequence alignment was also analyzed by the neighbor-joining distance method (MEGA, Kumar et al. 1993). The topology of the NJ tree (Fig. 3) was similar to the PAUP tree.

Discussion

ITS sequence phylogeny of the Pooideae

The phylogenetic relationships of the Pooideae (Fig. 2, 3) inferred from the ITS sequences showed that each tribe is monophyletic. The tribal boundaries of all taxa, except for the Aveneae and the Poeae, were well defined on the ITS trees. The majority rule consensus tree from parsimony analysis placed the Meliceae as the basal clade in the subfamily. One of the three shortest trees clustered the Meliceae and Stipeae as sister groups of a monophyletic clade. In contrast, the NJ tree showed that the Stipeae is basal and that it forms a basal paraphyletic assemblage with the Meliceae and Brachypodieae (Fig. 3). One unexpected result was that both parsimony and distance analyses consistently placed the Brachypodieae as the sister group to a monophyletic clade consisting of the Poeae, Aveneae, Bromeae and Triticeae. The Poeae/Aveneae and Bromeae/Triticeae clades are sister groups. The topologies of PAUP and NJ trees are comparable except in their basal taxon (Meliceae or Stipeae). However, the NJ

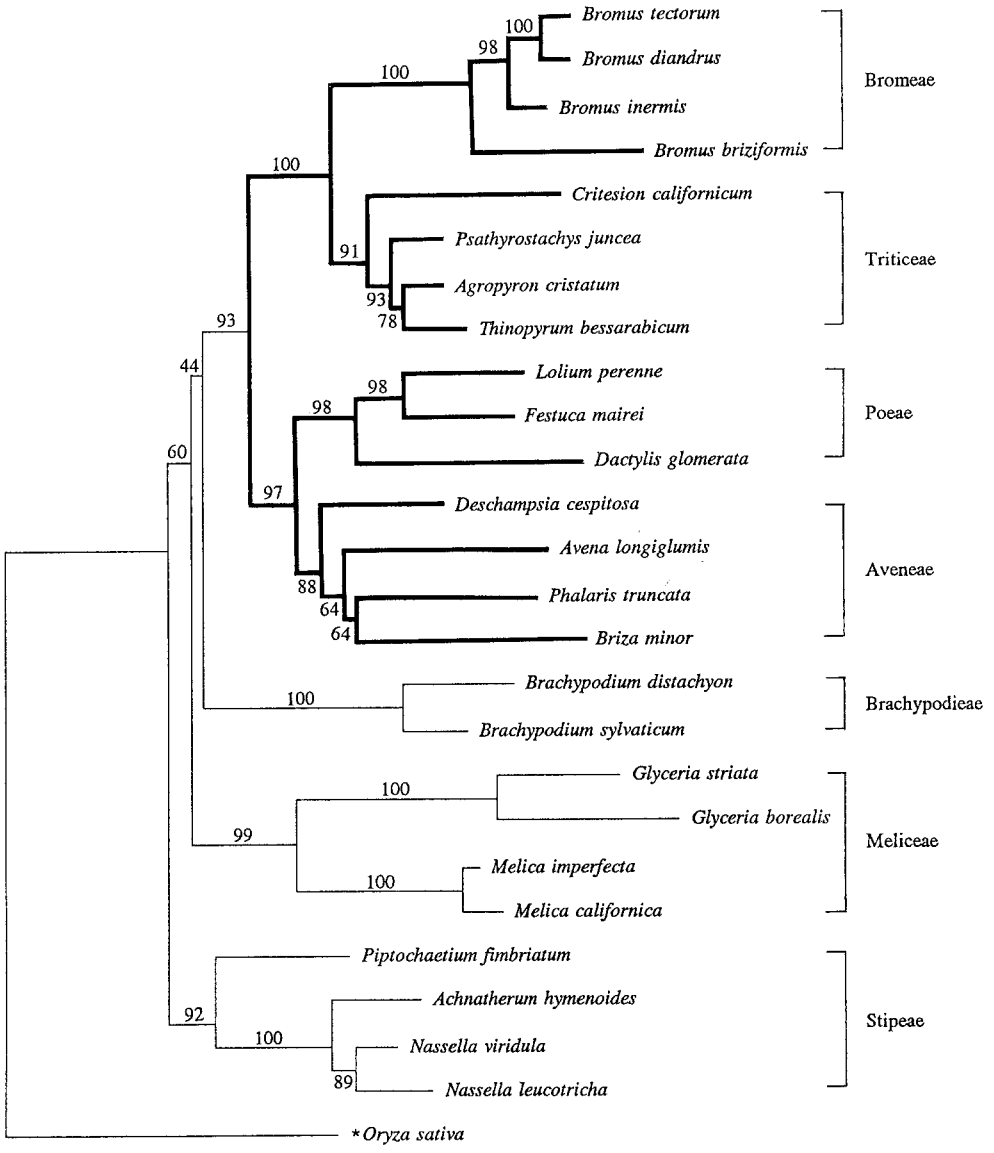


Fig. 3 Neighbor-joining tree using a Kimura 2-parameter distance. The *Asterisk* designates the outgroup species. *Numbers* on the branches are bootstrap values from 500 replicates of NJBOOT analyses (MEGA). Branch lengths are proportional to distance. *Bold lines* indicate the pooid "core" clade

tree seems to be better defined than the PAUP tree, despite the uncertainty in the basal clade. An extended pooid data set of ITS sequences may redefine the basal clade. Meanwhile, the Stipeae is preferred as the basal clade. As it is broadly defined, the Pooideae is morphologically a heterogeneous clade. Some of the Stipeae species possess morphological characters which indicate a link between the pooids and the bambusoids, e.g., the basic chromosome number, $x = 12$, the occurrence of three lodicules, and the presence of microhairs. The accumulation of starch instead of fructans by the stipoids may also hint at a connection with the bambusoids.

ITS phylogeny and chromosomal evolution

ITS sequence-based phylogeny identifies a monophyletic pooid "core" clade consisting of the Poeae, Aveneae, Bromeae and Triticeae, all containing large chromosomes with a basic number of $x = 7$. The Brachypodieae is distantly related to the Bromeae and Triticeae, but is closely related to the Meliceae and Stipeae, all with much smaller chromosomes (except the genus *Melica* with secondary derived larger chromosomes) and with basic chromosome numbers more than $x = 7$ (Brachypodieae, $x = 7$, 9; Meliceae, $x = 8$, 9 and 10; Stipeae, $x = 10$, 11 and 12). The ITS phylogeny of the seven pooid tribes is in close agreement with the direction of chromosome evolution in grasses, i.e., a reduction in the number, and an increase in the size, of chromosomes (Avdulov 1931; Stebbins 1956; Sharma 1979; Stebbins 1982, 1987). The ITS sequence data and cytogenetic evidence indicate that large chromosomes with a basic number of $x = 7$ evolved only once among the tribes in the Pooideae and pre-dated the divergence of the Poeae, Aveneae, Bromeae and Triticeae.

Comparisons between ITS and cpDNA phylogenies

The topologies of the ITS and cpDNA (Soreng et al. 1990) trees have similar tribal boundaries. The branch-

ing patterns within the tribe of these two trees could not be directly compared because only the Meliceae contained a few comparable taxa. One unexpected result was the position of the Brachypodieae whose divergence preceded the separation of the Poeae, Aveneae, Bromeae and Triticeae on the ITS tree, but was placed in the Bromeae and Triticeae clade based on cpDNA data (Soreng et al. 1990). It is not known whether this disagreement in the position of the Brfachypodieae between the ITS and cpDNA trees reflects differences in nucleotide substitution rates between the two genomes or other factors. In an analysis of the restriction-site variation of the cpDNA of extended taxa, the Brachypodieae is placed with the Meliceae (Davis and Soreng 1993), which differs from the conclusion of Soreng et al. (1990).

In an independent study, P. Catalan and R. G. Olmstead have sequenced a portion of the *ndhF* gene region of the cpDNA of 30 similar pooid taxa. Their results are highly concordant with our ITS sequences data especially concerning the position of the Brachypodieae (Catalan P, and Olmstead R. G., in preparation).

Comparisons between molecular and morphological phylogenies

All three independent molecular phylogenies are generally in agreement and identify the distinct pooid "core" clade which includes the Poeae/Aveneae and the Bromeae/Triticeae, but are at odds with the morphological phylogeny reported by Kellogg and Watson (1993) which has no resolution and fails to find a hierarchical structure. The disagreement between molecular and morphological phylogenies could not be explained by a rapid burst of radiation or extensive gene flow since the molecular phylogenies of both nuclear and chloroplast genomes are congruent. The only interpretation for this unusual fact is the true parallelism and mosaic evolution in morphological characters in grasses that have long been recognized by Stebbins (1982, 1987), Clayton (1981), and Clayton and Renvoize (1986). We also found this kind of incongruence in Triticeae phylogenies (Hsiao et al., 1995). Phylogenies based on molecular and morphological data are often congruent in plants (Donoghue and Sanderson 1992). Whether this phenomenon is unique to the grass family as a whole must be tested on other grass subfamilies. When contrasting information exists, true species phylogeny can only be determined by evaluating all the available relevant evidence, e.g., cytogenetic evidence, biogeographical information, and physiological data.

ITS phylogeny and systematic treatment

Based on numerical analyses of morphological and antomical data (Macfarlane and Watson 1980, 1982; Watson et al. 1985; Macfarlane 1987) the Pooideae was divided into two supertribes, the Poodae and the Triticodae. These authors excluded the Stipeae from the Pooideae. The Meliceae was placed in the Poodae because it is linked to Poeae by compound starch grains. *Bromus, Brachypodium* and the Triticeae are linked by characters such as simple starch grains, linear hila, hairy ovaries, straight awns and large spikelets. One major character shared by the Brachypodieae and Triticeae is a racemose inflorescence.

ITS phylolgeny depicted two major groups of the Pooideae: the basal assemblage includes the Stipeae, Meliceae and Brachypodieae while the monophyletic pooid "core" clade includes the Poeae, Aveneae, Bromeae and Triticeae. These two groups are distinct and consistent on all the molecular trees and are well supported by cytogenetic evidence. However, the hierarchical positions of the Meliceae and Brachypodieae in the subfamily based on molecular data are quite different from their positions in the numerically based pooid classification. The Meliceae (except genus *Melica*) was the only member in the Poodae with small chromosomes and a base chromosome number more than $x = 7$; as was the Brachypodieae in the Triticodae.

We included the Stipeae in our pooid study based on our preliminary ITS sequences data of the whole family which indicated that the Pooideae is monophyletic and includes all seven tribes as well as the Nardeae, Lygeeae, and Brachyelytreae (Hsiao et al., unpublished data). The Stipeae as excluded from the subfamily by Macfarlane and Watson (1980, 1982), Barkworth (1982), Watson et al. (1985), Barkworth and Everett (1987), and Macfarlane (1987), but was included by Stebbins and Crampton (1961), Clayton and Renvoize (1986), Kellogg and Campbell (1987), and Soreng et al. (1990). Based on the molecular phylogenies, the Stipeae should not be the only tribe to be excluded from the Pooideae. It might be better also to exclude the Meliceae and the Brachypodieae, thereby creating a narrowly defined monophyletic pooid clade consisting exclusively of taxa with large chromosomes and a basic number of $x = 7$. Molecular data and cytogenetic evidence do not support the erection of two supertribe divisions in the Pooideae; namely, the Poodae (Poeae, Seslerieae, Aveneae, Meliceae) and the Triticodae (Triticeae, Brachypodieae, and Bromeae).

Conclusions

The ITS sequence-based phylogeny of the Pooideae has a consistent hierarchical structure that is well supported by cytogenetic evidence (Avdulov 1931, Stebbins 1956, 1982, 1987) but differs from recent classifications (Macfarlane and Watson 1980; Macfarlane 1987) and a morphology-based phylogeny (Kellogg and Watson 1993). The close agreement between phylogenies of the nuclear and the choloroplast genomes (Soreng et al 1990; Davis and Soreng 1993; Catalan and Olmstead, personal com-

munication) reaffirm our confidence in molecular phylogeny. These molecular data, hopefully, will provide grass systematists with useful information for making taxonomic decisions.

Acknowledgements We thank T. H. Hsiao for assistance in phylogenetic analyses; M. E. Barkworth, S. L. Hatch, E. A. Kellogg, and P. McGuire for their valuable comments on the manuscript. We also thank M. Curto for identifying and collecting some of the plant samples.

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