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CHLOROPLAST-DNA PHYLOGENETICS AND BIOGEOGRAPHY IN A RETICULATING GROUP: STUDY IN POA (POACEAE)[1]

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Cladistic analysis of *Poa* chloroplast DNA (cpDNA) restriction sites tested previously hypothesized relationships within the genus. Forty-six taxa representing 19 sections or groups and three subgenera of *Poa* and two out-group genera, *Puccinellia* and *Bellardiochloa,* are analyzed. Five major and several minor cpDNA groups are identified. The cpDNA cladogram is generally congruent with the subgeneric taxonomy of *Poa.* Exceptions are reclassified or are discussed in terms of character incompatibilities and possible reticulation events. The cpDNA tree detected relationships among sections that were unresolved using traditional character sets and provides a basis for polarization of morphological character states. An assessment of biogeographic events based on the cpDNA tree suggests: 1) *Poa* originated in Eurasia; 2) at least six groups of species independently colonized North America; and 3) two of the latter groups colonized South America, and one closely related group colonized New Zealand and Australia. The cpDNA tree provided a conservative estimate of the number of amphi-neotropical disjunctions when compared to the known number of species disjunctions.

Species of the genus *Poa* are native to arctic, temperate, and high-elevation-tropical regions around the world. The genus is diverse, including some 500 species that occur in a wide range of habitats. Although taxonomically difficult, and historically a catch-all, the genus as delimited today is considered by most agrostologists to be morphologically coherent. Clayton and Renvoize (1986, p. 101) in their revision of all grass genera concluded that "*Poa* is an extremely uniform genus for which there is no satisfactory infrageneric classification. Its taxonomy is rendered difficult by the dearth of useful discriminatory characters and complicated by the widespread occurrence of apomixy and introgression." Bor (1952, pp. 7, 8) prefaced a major work on *Poa* with, "The systematic treatment of the species . . . is one of the most bewildering and difficult of taxonomic studies. While many species are clear-cut and can be recognized at a glance, there are groups of species about which one can only conclude that their evolutionary history has been so complex that they do not lend themselves to systematic treatment by present taxonomic methods. One cannot rely upon a single character to separate species in such groups, but combinations of more or less variable characters must be used. . . ." Stebbins (1950, p. 405) stated that, "when this genus is better known, it may have to be regarded as a single huge polyploid complex, which is in part purely sexual, in part facultatively apomictic, and which contains in addition obligate apomicts." Such assertions leave considerable doubt as to whether any satisfactory, much less phylogenetic, classification can be achieved.

Although subgeneric treatments and species alignments in *Poa* have converged (Edmondson, 1978, 1980 [Europe]; Nicora, 1978 [South America]; Tzvelev, 1983 [USSR]; Soreng, 1985 [North America]), higher level reltionships are rarely based on explicit hypotheses of character evolution. I have found that many morphologically distinguishable groups of *Poa* are also coherent in their ecological tolerances and breeding system characteristics. However, character states used to unite groups or infer cladistic relationships mostly are continuous rather than discrete, and often are inconstant.

The main objective of this paper is to compare a cladistic analysis of chloroplast DNA (cpDNA) restriction sites (RS) with current subgeneric classification. A second objective is to use cpDNA RS as markers of geographic radiation.

As an independent test of phylogeny where

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relationships may be reticulate, analysis of cpDNA RS offers advantages (Palmer, 1987). The slow evolution of chloroplast genomes provides phylogenetic resolution at high taxonomic ranks independent of previously hypothesized character transitions (except for out-group choice). Uniparental (predominantly maternal) inheritance and absence of intermolecular recombination disallow reticulation in cpDNA phylogenies, as opposed to morphological, plant mitochondrial-DNA, or nuclear gene phylogenies. The possible inlfuence of hybridization in the species phylogeny can be evaluated from congruence between cpDNA cladograms and traditional species groups, and by subsequent experimentation (e.g., by isozyme analysis). The present study provides an evaluation of congruence with current classification and morphological data.

A recent phylogenetic analysis of cpDNA RS in the subfamily Pooideae (including, among other genera; *Arctagrostis, Bellardiochloa, Briza, Catabrosa, Chascolytrum, Dactylis, Festuca, Microbriza, Puccinellia, Sclerochloa, Sesleria,* and *Torreyochloa*) suggested *Poa* is monophyletic (Soreng, Davis, and Doyle, in press). In this study, infrageneric relationships of *Poa* are assessed using *Bellardiochloa* and *Puccinellia* as out-groups. Traditionally recognized taxa were mostly supported, although some are in need of reevaluation in light of new cpDNA data. Several instances of putative reticulate evolution of chloroplast genomes are revealed. The cpDNA cladogram was consistent with a biogeographic scenario involving the origin and primary diversification of *Poa* in Eurasia; spread of at least six groups to North America, two of which occur in South America. It also supports a close relationship between the major New World group and a group occurring in Australia and New Zealand.

MATERIALS AND METHODS

The sample—The 46 taxa examined are listed in Table 1, along with chromosome numbers, mode of reproduction, and classification. Sampling was designed to include sexually reproducing species from as many diploid taxa as possible, as many sections as available, and duplicates within sections or groups if possible. Species were also selected to test hypotheses of the origin of dioecy and geographic connections of New World *Poa*. Several known apomictic species were included in order to learn more about their origins. In four cases cpDNA was in short supply and different accessions were pooled (*Poa cusickii 2977, 2993, 2994*; *P. fendleriana* subsp. *fendleriana*; *P. f.* subsp. *albescens*; *P. labillardierei* and *P. sieberiana* [two closely related Australian species]). In three cases RS data from different accessions of the same species were pooled (after checking for synapomorphies) to make complete sets (*P. arctica, P. cusickii, P. pratensis*). All other terminal taxa represent a single individual or population or adjacent local populations. Multiple accessions were screened for intraspecific variability of RS in *P. arctica, P. cusickii, P. fendleriana,* and *P. pratensis.* Seven samples of *P. secunda* sensu lato (s.l.) were checked.

I describe the sampling of species in some detail as it is critical to the interpretation of the results. Diploids are included from four of 11 sections in which they are known. Among the polyploids, *Poa annua* (Ochlopoa) is an infrasectional allopolyploid (Tutin, 1957). Morphological comparisons suggest that the apomicts, *Poa alpina, P. arctica, P. chamaeclinos, P. palustris,* and *P. nemoralis* are also derived from within their respective sections. Other apomicts sampled are likely to involve wider (intersectional) parentage; *P. bulbosa, P. compressa, P. pratensis, P. wheeleri* (Soreng, 1986), and *P. secunda* s.l. Samples of the remaining species were from putatively sexual populations, except in *P. fendleriana* subsp. *fendleriana,* where one sexual and two apomictic populations were pooled (Soreng, 1986).

The breadth of taxonomic variation sampled in the genus is of concern. In this regard, all but two monotypic (Leptophyllae, Nanopoa) and one small section (Nivicolae) of *Poa* currently recognized in Europe and the USSR were sampled. In North America I have sampled all of the major and most of the minor groups of *Poa.* From South America I have sampled two major groups and two minor groups (the latter sampled from Mexico), leaving an antarctic island group (*P. flabellata* (Lam.) Raspail, *P. cookii, P. rammossisima* Hook. f.) and the small subgenus Andinae s.s. (which is questionably distinct from group IVB species) unsampled there. A regional gap exists in Southeast Asia both in the sample and in our knowledge of sectional affinities of the species. However, I believe that many if not most species of that region can be accommodated within sections found in the USSR. My estimation, derived from a survey of the Gray and U.S. National Herbarium collections and the literature, is that species have been sampled from three-quarters of the sectional level groups in the genus.

Two outgroups were used. *Puccinellia distans,* a polyploid, has restriction patterns that are nearly identical to those of other species of that genus, including diploids (Davis and So-

TABLE 1. *Chloroplast groups,[a] taxonomic affiliations,[b] chromosome numbers, breeding systems, and source information[c] for species[d] of* Poa *and out groups from which cpDNA RS were analyzed*

Genus (number of species) Subgenus (number of species) Section, subsection or group (number of species) Species Taxon no.	Chromosome number (2*n*)	Chloroplast group Breeding system	Accession	Geographic distribution Source and origin
		Chloroplast out-groups		
Puccinellia Parl. (80–120)				World-wide
1. *P. distans* (L.) Parl. cv. Fults	28–42	self-comp.	PUDI5591	12 USA, Washington (introduced)
Bellardiochloa Chiov. (2–5)				E. Europe
2. *B. violacea* (Bellardi) Chiov.	14	self-incomp?	253455	13 Yugoslavia
Poa L. (500)				
		Chloroplast group I		
Subgenus *Arctopoa* (Griseb.) Prob. (5)				
Arctopoa (1–2)				Boreal, NE. Asia, N. America
3. *P. eminens* J. S. Presl	42	self-incomp?	J85-73	6 Canada, Hudson Bay
Subgenus *Poa* (490)				
"*Sylvestres*" (6)				Eastern N. America
4. *P. alsodes* A. Gray			3350	5 USA, Virginia
5. *P. saltuensis* Fern. & Wieg.	28		3346	5 USA, Virginia
"*Sylvestres*"?				Eastern N. America
6. *P. autumnalis* Muhlenb. ex Elliott	28	self-comp?	3205 & 7	5 USA, Virginia
		Chloroplast group II		
Poa subsect. *Caespitosae* V. Jir. [Bulbophorum (Asch. & Graebner) V. Jir.] (3)				Europe & Circumboreal
7. *P. alpina* L.	21–56	apomixis	POAL4094	12 Canada
		Chloroplast group III		
Ochlopoa (Asch. & Graebner) V. Jir. (5)				Mediterranean
8. *P. annua* L.	28	gynomonecy self-comp.	sn.	14 USA (introduced)
		Chloroplast group IVA		
Poa (25)				Eurasia & Circumboreal
9. *P. arctica* R.Br.	56	apomixis	1094	2 Canada, Alberta
P. articia	56–59	apomixis	1142	2 USA, Montana
10. *P. pratensis* L.	42–98	apomixis	POPR4713	12 USA, Washington (introduced)
P. pratensis			2938	1 USA, California (introduced)
Macropoa F. Herm. ex Tzvelev (7)				Asia
11. *P. iberica* C. Fischer & C. Meyer	28		325462	13 USSR
		Chloroplast group IVC2		
"*Dioicopa*" E. Desv. (previously accorded subgeneric status) (45)				N. & S. America (New Zealand?)
12. *P. arachnifera* Torrey	42–84	dioecy	sn.	7 USA, Texas
13. *P. iridifolia* Hauman	28	dioecy	284254	13 Argentina
14. *P. lanigera* Nees	28	dioecy	sn.	10 Argentina

TABLE 1. *Continued*

Genus (number of species) / Subgenus (number of species) / Section, subsection or group (number of species) / Species / Taxon no.	Chromosome number ($2n$)	Chloroplast group: Breeding system	Accession	Geographic distribution: Source and origin
		Chloroplast group IVB		
"*Australopoa*" (42 at least)				Australia, New Zealand
15a. *P. labillardierei* Steudel		self-incomp?	282383	13 Australia
15b. *P. sieberiana* Sprengel		self-incomp?	263863	13 New Zealand
		Chloroplast group IVC1		
"*Dasypoa*" Pilger (originally proposed as a genus) (2–3)				Central and South America
16. *P. conglomerata* Rupr.		self-comp.	3315	1 Mexico, Mexico
Diversipoa Chrtek & V. Jir. (20)				N. & S. America
17. *P. bigelovii* Vasey & Scribner	28	self-comp.	2110b	4 USA, New Mexico
18. *P. occidentalis* Vasey	14	self-comp.	3353	1 USA, New Mexico
19. *P. orizabensis* A. Hitchc.		self-comp?	3314	1 Mexico, Mexico
Homalopoa Dumort. (6)				Eurasia
20. *P. hybrida* Gaudin	14		249765	13 Greece
"*Nervosae*" (North American gynodioecious *Poa*) (6)				Temperate N. America
21. *P. cuspidata* Nutt.	28	partial-gynodioecy	3302 & 3	1 USA, Virginia
22. *P. nervosa* (Hook.) Vasey	28 + 1	partial-gynodioecy	2960	1 USA, Oregon
23. *P. rhizomata* A. Hitchc.	28	sub-dioecy	2924 & 5	1 USA, California
24. *P. strictiramea* A. Hitchc.	28 + 1	partial-gynodioecy	2304	2 Mexico, Chihuahua
25. *P. tracyi* Vasey	28 + 1	partial-gynodioecy	383 & 5	1 USA, New Mexico
"*Punapoa*" (10–20)				S. American Andes, Mexico
26. *P. candamoana* Pilger		gynomoecy	478580	13 Peru
27. *P. chamaeclinos* Pilger		gynomonecy	3315	1 Mexico, Mexico
"*Madropoa*" (North American dioecious *Poa*) (8)				Temperate N. America
28. *P. confinis* Vasey	42	sub-dioecy	2958	1 USA, Oregon
P. cusickii Vasey				
29a. ssp. *cusickii*	28	sub-dioecy	2995	1 USA, California
29b. ssp. *cusickii*			2977, 2993, 2994	1 USA, Oregon & California
P. fendleriana (Steudel) Vasey				
30. ssp. *albescens* (Scribner & T. Williams) Soreng	28 + 1	dioecy	1780, 2305, 2309	2 Mexico, Chihuahua
31. ssp. *fendleriana*	56	dioecy	2170, 2180, 2181	1 USA, New Mexico
32. *P. piperi* A. Hitchc.	28	dioecy	2921, 2954	1 USA, California & Oregon
"*Madropoa* × *Nervosae*" (*P. cusickii* spp. *cusickii* × *P. nervosa*)				
33. *P. wheeleri* Vasey	56–91	apomixis	3356	1 USA, Montana
		Chloroplast group VD		
Poa subsect. *Bulbosa* V. Jir. [Bulbophorum (Asch. & Graebner) V. Jir.] (10–15)				Europe, Middle East
34. *P. bulbosa* L.	21–42	apomixis (bulbiferous)	5128183	9 USA, California (introduced)
"*Secundae Halophytae*" (2)				Western N. America

TABLE 1. *Continued*

Genus (number of species) / Subgenus (number of species) / Section, subsection or group (number of species) / Species / Taxon no.	Chromosome number ($2n$)	Chloroplast group: Breeding system	Accession	Geographic distribution: Source and origin
35. *P. napensis* Beetle	42	sexual?	2926	1 USA, California
"*Secundae Secundae*" (3)				Western N. & S. America
P. secunda J. S. Presl sl.				
[*P. ampla* Merr.]	62–71(–100)	apomixis	sn.	15 USA
36. [*P. canbyi* (Scribner) Howell]	72–105	apomixis	1749-7	1 USA, New Mexico
37. [*P. canbyi* cv. Canbar]			POCA4578	12 USA, Washington
[*P. nevadensis* Vasey]	62–70	apomixis	5101	6 USA,
[*P. nevadensis* Vasey]				16 USA,
38. [*P. sandbergii* Vasey]	74–87	apomixis	POSA3273	12 USA
39. *P. tenerrima* Scribner	42	sexual	2933	3 USA, California
		Chloroplast group VE		
Abbreviatae Nannf. ex Tzvelev (6)				Circumboreal, Berringia
40. *P. brachyanthera* Hultén	14	self-comp?	2231	1 USA, Alaska
41. *P. keckii* (Soreng ined.)		self-comp?	3363	1 USA, California
Oreinos (Asch. & Graebner) V. Jir. (7)				Circumboreal
42. *P. paludigena* Fern. & Wieg.		self-comp.	3221	5 USA, Virginia
P. laxa subsp. *fernaldiana* (Nannf.) Hylander	42	self-comp.	3401	1 USA, Vermont
Stenopoa Dumort. subsect. *Tricopoa* (Asch. & Graebner) Maire (2)				Eurasia
43. *P. compressa* L.	42–56	apomixis	POCO4247	12 USA, Washington (introduced)
P. compressa cv. Rubens			40, 216	11
Stenopoa Dumort. subsect. *Stenopoa* (25)				Circumboreal & Eurasia
44. *P. nemoralis* L.	28–56	apomixis	11-7-1987	8 West Germany
45. *P. palustris* L.	28	apomixis	3354 & 5	1 USA, Montana (introduced)
Coenopoa Hylander (1–2)				Europe
46. *P. trivialis* L.	14	self-incomp.	POTR2128	12 Denmark

[a] "Chloroplast groups" of species are defined by restriction site analysis and correspond to Fig. 2.

[b] Taxonomy follows Tzvelev (1983), Edmondson (1980), and Soreng (1985, 1986) with informal groups in quotations.

[c] Source codes are 1 = R. J. Soreng (RJS), 2 = RJS & R. Spellenberg, 3 = RJS & G.L. Stebbins, 4 = RJS & D. Ward, 5 = RJS & T. Weibolt, 6 = S. J. Darbyshire, 7 = J. Reed, 8 = H. Scholz, 9 = L. Wagner, 10 = Instituto Nacional de Techologia Agropecuria Buenos Aires, 11 = Jacklan Seed Co., 12 = Native Plants Inc., 13 = USDA Northwest Regional Plant Introduction Station, 14 = O. M. Scott Co., 15 = Sharp Bros. Seed Co., 16 = J. I Davis.

[d] Species code numbers correspond to those used in Tables 2, 3 and Fig. 1.

reng, unpublished data). *Bellardiochloa violacea* is a diploid member of a small genus included within *Poa* by Clayton and Renvoize (1986), but not by Edmondson (1978), Tzvelev (1983), or Soreng, Davis, and Doyle (in press).

DNA isolation and RS analysis—Purified cpDNA was isolated from species listed in Table 1 using minor modifications (Soreng, Davis, and Doyle, in press) of a nonaqueous extraction procedure (Dally and Secund, 1989) or modifications (Hilu, 1988) of a sucrose gradient method (Saltz and Beckman, 1981). The latter method usually yielded 5–10 μg of sufficiently pure cpDNA for restriction work per 30–100 g of fresh tissue. Although some degradation of the DNA was apparent with both methods, higher (0.5–1 μg/g) and more consistent yields were obtained by the nonaqueous extractions using only 2–15 g fresh weight of leaves. The latter procedure was particularly successful in liberating cpDNA from mature, sclerified material.

Digests were performed according to Bethesda Research Labs' (BRL) instructions using 14 enzymes that recognize six base pair (bp) sequences: *Bam*H I, *Bcl* I, *Bgl* II, *Cla* I, *Eco*R I, *Hin*d III, *Hpa* I, *Kpn* I, *Pst* I, *Pvu* II, *Sal* I, *Sca* I, *Sma* I, and *Xho* I. Digested DNAs were size fractionated electrophoretically in agarose (1.2–1.0% for frequent cutters, 0.7–0.5% for rare cutting enzymes). DNA was transferred from gels to Zetaprobe nylon membranes (BRL) for probing by the methods of Southern (1975) or Reed and Mann (1985). Probing was performed using cloned fragments of the *Pennisetum americanum* cpDNA library (Thomas et al., 1984) as reported by Soreng, Davis, and Doyle (in press).

Restriction digest patterns were diagrammed from photos of the ethidium bromide stained gels and fragment sizes calculated from lambda *Hin*d III or *Pst* I standards. The general order of single digest fragments was indicated by their homology with specific *Pennisetum* cpDNA probes. Fragments hybridizing to adjacent probes, and subsequent RS changes among cpDNA fragments of different species within probe regions, were used to arrange fragments in linear order. The entire chloroplast genome was mapped in this manner for *Hin*d III, *Hpa* I, *Kpn* I, *Pst* I, *Pvu* II, *Sal* I, and *Sma* I (Fig. 1). For the remainder of the enzymes, RS were localized within and between probe regions (Table 2). Possible insertion/deletion (I/D) events (fragments less than 300 bp in length were not usually resolved on gels) were not included in the formal cladistic analysis, but their presence was scored (Table 3) for comparison with the RS cladistic structure.

Shared RS were scored as presence/absence data (Fig. 1) and analyzed cladistically by implicit-enumeration of all most parsimonious trees using HENNIG-86 (Farris, 1988). Dollo parsimony (PHYLIP 3.0, "Dollop" option: Felsenstein, 1987) was invoked to examine the effects on tree structure of excluding parallel RS gains. Shortest trees generated by each method were analyzed with CLADOS (K. Nixon, unpublished computer program) to examine the distribution and homoplasy of RS, investigate alternative trees, and produce cladograms.

RESULTS

Forty-four taxa of *Poa* from 19 sections and groups, distributed in three of four traditional subgenera, and two outgroups were examined for RS variation using 14 restriction enzymes. Of some 16,000 RS examined, about 400 are distinct (constituting circa 0.5% of the chloroplast genome), 127 vary within the study group (Fig. 1), and 43 are synapomorphic within *Poa*. Approximate chloroplast coordinates (relative to the *Oryza* sequence) of RS are presented for some restriction enzymes (Fig. 1); other RS were localized within probe regions (Table 2).

The RS presence/absence data (Fig. 1) were analyzed in a cladistic framework. Eleven equally parsimonious cladograms were found. Excluding autapomorphies, these are 87 steps long with a consistency index (C.I.) of 0.84. One of these trees, that which most closely agreed with the strict consensus tree, is presented in Fig. 2. Alternative trees are discussed below. Sixty-one events scored as unique I/D or RS that produced undetected small fragments (Table 3) are generally congruent with the cladogram and add support to the recognition of certain groups.

A second cladistic analysis was performed using *Poa eminens* as an out-group within *Poa*, as is suggested by Tzvelev's (1983) treatment of the genus. This resulted in ten trees with essentially the same structure as Fig. 2 that were (excluding autapomorphies) 55 steps long with a C.I. of 0.91. This demonstrates that about half of the homoplasy evident in Fig. 1 resulted from the inclusion of the out-group genera.

The major structure of the cpDNA phylogeny of *Poa* is relatively robust. Out-group analysis using data from seven restriction enzyme (RE) digests demonstrated *Poa* to be monophyletic (see introduction). The cladistic struc-

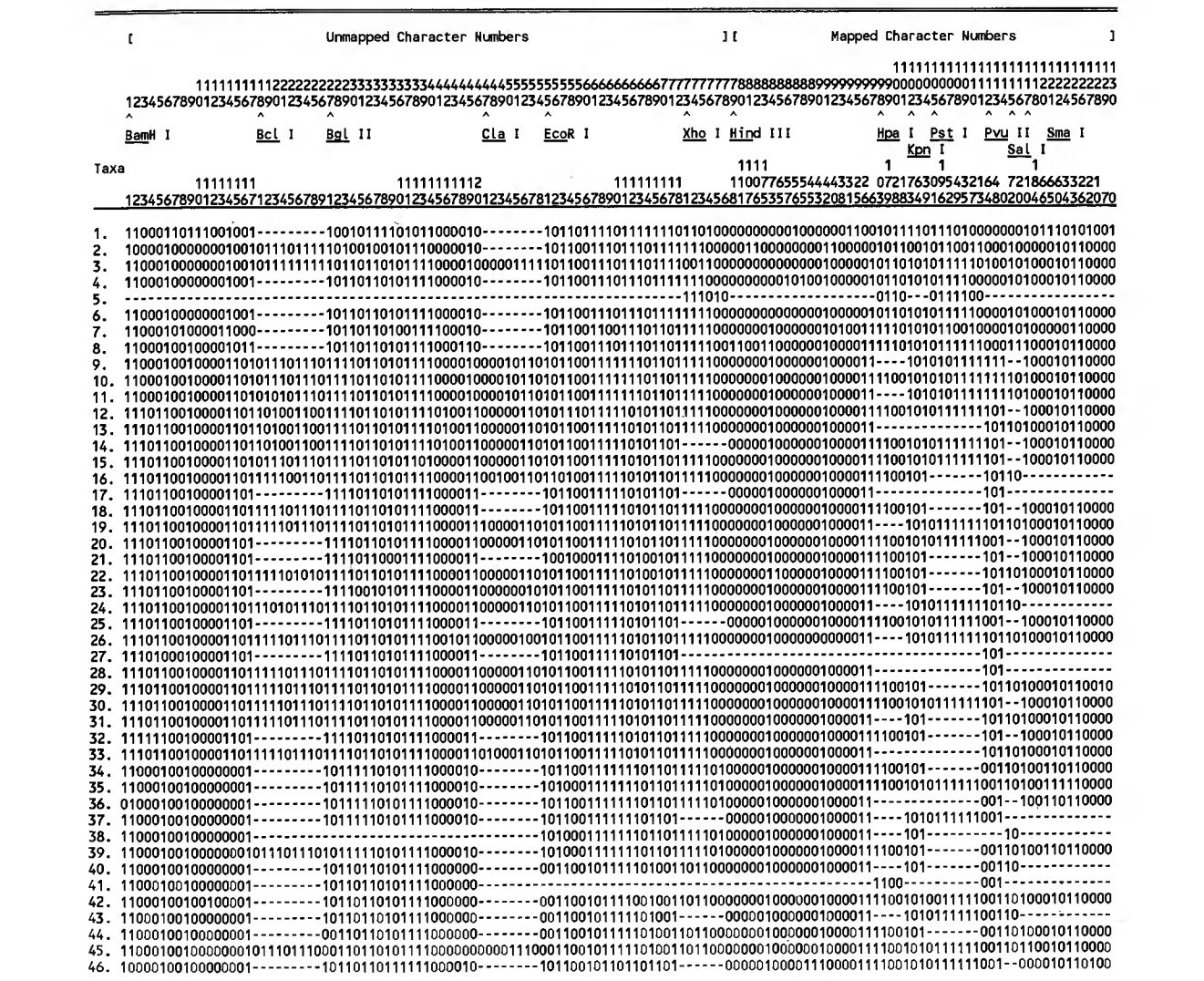

Fig. 1. Apomorphic restriction sites among species of *Poa* and out-groups. Taxa numbered as in Table 1. RS arranged by enzyme; character numbers correspond to Fig. 2; RS arranged within probe regions numbered as in Table 2; mapped RS coordinates correspond to approximate order relative to the *Oryza* cpDNA sequence, beginning at the junction of the large single copy region with the inverted repeat nearest *psb*A gene and proceeding toward the *rbc*L gene. Data codes are: 0 = absent, 1 = present, − = no data. A ∧ marks the first character in each enzyme RS set.

ture within *Poa* did not change when the two data sets were merged, but fewer shortest trees were obtained. Based on these data it was not possible to resolve the trichotomy between *Poa, Puccinellia,* and *Bellardiochloa,* in strict consensus trees. Within *Poa,* three basal groups (I–III) and two major derived cpDNA sister groups (IV, V) were identified. Groups IV and V share three to four (character 26 is not fixed in position) derived RS and four I/D events, but are separated by five RS and 14 I/D events. Dollo parsimony, which prohibits parallel gains, resulted in two changes within *Poa*: placement of *P. alpina* as a sister group to the rest of *Poa* and fixation of the branching pattern in group IV as depicted in Fig. 2. However, the five major groups with their included species remained unchanged in all analyses.

The following sections or groups of *Poa* are represented within the five major cpDNA groups. Group I: *Arctopoa, Sylvestres*; Group II: *Caespitosae*; Group III: *Ochlopoa*; Group IV: *Australopoa, Dasypoa, Dioicopoa* sensu stricto (s.s.), *Diversipoa, Homalopoa, Macropoa, Madropoa, Nervosae, Poa, Punapoa*; Group V: *Abbreviatae, Bulbosae, Coenopoa, Oreinos, Secundae, Stenopoa, Tricopoa.* Most of the variation among the parsimony trees

TABLE 2. *Unmapped* Poa *chloroplast DNA restriction site changes*

No.[a]	Enzyme	Region[b]	Change[c]	Taxa[d]
1	*Bam* HI	7	1.95 + 0.64 → 2.6	36
2		5	8.1 + 1.3 → 9.3	2, 46
3		5	4.65 → 0.67 + 4.0	12–33
4		5	4.0 → 2.2 + 1.7	32
5		5	3.5 → 0.5 + 3.0	12–33
6		5	3.0 + 4.4 → 7.5	27
7		5	4.4 → 2.2 + 2.3	1
8		5	4.4 → 3.6 + 0.8	7
9		5-4	17.5 + 2.5 → 20.1	2–7
10		4	17.5 → 4.4 + (6.4 + 4.7)	1
11		4-3	17.5 → (4.4 + 6.4) + 4.7	1
12		4-3	17.5 → 13.0 + 4.45	42
13		3	1.7 → 1.4 + 0.4	7
14		3-6	2.6 + 1.05 → 3.9	34–46
15		2	6.65 → 0.76 + 5.7	9–33
16		2	6.7 → 3.4 + 3.5	8
17		2	6.7 + 3.45 → 10.1	7
18	*Bcl* I 7	6	2.9 → 1.75 + 1.25	12–14, 16–33
19		5	2.2 + 2.05 → 4.25	12–14
20		5-4	4.3 + 7.5 → 11.8 (partial?)	11, 24
21		3	2.5 + 1.84 → 4.5	12–14
22		3	1.84 → 0.88 + 0.95	3
23		3	1.84 + 6.2 → 8.75	16
24		3-2	1.75 + 3.1 → 4.9	22
25		3-2	3.1 + 2.5 → 5.6	12–14
26		2-1	3.55 + 6.9 → 10.45	(7–)9–46
27	*Bgl* II	1	5.5 + 4.0 → 9.6	44, 45
28		7	2.9 → 2.6 + 0.3	9–33
29		6	4.1 + 0.8 → 4.8	1
30		5	0.8 + 7.7 → 8.6	2
31		5	7.7 → 5.7 + 2.0	34–39
32		5	0.7 + 6.9 → 7.7	23
33		5	6.9 → 1.5 + 5.5	2
34		5	6.9 → 2.4 + 4.1	1
35		5-4	6.9 + 3.1 → 10	21
36		4	3.1 → 2.4 + 0.7	46
37		4	3.5 + 1.78 → 5.4	7
38		3	1.6 + 1.2 → 2.9	1
39		3	1.2 + 3.4 → 4.6	15
40		3	3.4 + 1.1 → 4.5	2
41		3-6	16.5 → 8.6 + 7.2	7
42		3-6	16.5 → 11.5 + 5.0	12–14
43		3-6	16.5 → 12.0 + 4.6	26
44		3-6	16.5 → 14.5 + 2.2	8
45		2	2.2 + 1.3 → 3.5	40–45
46		1	5.0 → 4.1 + 1.0	12–33
47	*Cla* I	5	12.5 → 11.55 + 0.95	19
48		5	3.95 → 1.95 + 1.85	33
49		4	8.0 → 5.35 + 2.55	16
50		3	5.0 → 4.1 + 1.0	9–11
51		3	0.7 + 1.03 → 1.75	9–33
52		2	0.83 + 1.45 → 2.35	23
53		2	1.5 + 3.45 → 1.18 + 3.77	26 (inversion?)
54		6	5.6 → 3.7 + 1.9	3
55	*Eco* RI	7	1.3 (IR) + 0.1 & 0.18 → 1.58 & 1.4	40–45
56		7	2.6 → 1.8 + 0.8	16
57		7	1.07 + 0.3 → 1.37	16, 21
58		7	2.6 + 0.4 → 3.0	35, 38–39
59		6	1.8 → 0.57 + 1.03	12
60		6	2.3 → 1.9 + 0.5	1
61		6	2.3 + 0.74 → 3.0	21
62		5	0.74 + 0.74 → 1.59	40–46
63		4	3.4 → 3.0 + 0.4	7
64		4	5.9 → 4.2 + 1.9	9–46
65		4	1.0 + 2.0 → 2.81	46

TABLE 2. *Continued*

No.[a]	Enzyme	Region[b]	Change[c]	Taxa[d]
66		4	2.0 + 0.3 → 2.31	12–33
67		3	6.8 + 1.0 → 8.0	42
68		3	6.8 + 1.9 → 5.8 + 2.6	1
69		3	1.8 + 1.28 → 3.1	21, 22
70		3	1.1 + 1.24 → 2.34	40–45
71		2	1.65 + 2.4 → 4.5	7–46
72		2	2.4 + 4.3 → 6.4	1
73		4	12.5 + 3 + 7.9 → 21.5	3
74		4	3.0 + 7.9 → 10.8	3, 40–45
75		3	9.6 + 7.6 → 17.6	1
76		2	3.9 + 16.5 → 20.4	2, 5–6
77		2	20.4 → 11 + 8.4	6
78		2	16.5 → 10.8 + 6.5	34–39

[a] RS are numbered in order by enzyme and probe region.

[b] Chloroplast probe regions are coded as follows with included map coordinates [increments = 1 kb, beginning at the juncture of the large single copy region near *psb*A and proceeding toward *rbc*L]: 1 = 0–12; 2 = 12–26; 3 = 25–43; 4 = 43–57; 5 = 57–78; 6 = 78–96, 117–135; 7 = 96–117.

[c] Changes are unpolarized; arrows indicate the state in the taxa listed.

[d] Taxa are numbered as in Table 1.

occurred in group IV. The structure of each of these groups is discussed below.

Groups I–III—The set of species included in these groups was constant, but the basal structure of the genus remains tentative. The *P. alpina* and *P. annua* accessions possessed many autapomorphies, suggesting independent divergences of their respective sections. The positions of these taxa were consistent among all global parsimony trees. However, as noted above, *P. alpina* moved to the basal-most position in *Poa* under the Dollo constraint. In all global parsimony trees, the sections *Sylvestres* and *Arctopoa* were depicted as a sister group to *P. alpina* and *P. annua* and groups IV and V. *Poa eminens,* although united with the *Sylvestres* by one RS loss, bears no apparent morphological, geographical, or ecological relationship to the group. *Poa eminens,* which occurs along arctic coastal-strand, is rhizomatous and halophytic (as are other species of subgenus *Arctopoa*), whereas *Sylvestres* are cespitose, temperate forest, montane species that share advanced characteristics of subgenus *Poa.* Although these sections shared one RS loss, this could represent parallelism (parallel losses being more frequent than parallel gains). Their otherwise similar RS patterns may stem in part from incomplete sampling in the latter group. Further resolution within cpDNA groups I–III requires additional RS analysis involving more species of *Poa* and more closely related genera.

Group IV—This group included three sharply defined subgroups; "A" (*Poa* and *Macropoa*), "C2" (*Dioicopoa*), arising from within or as a sister group to "C1" (a diverse group of species including *Dasypoa, Diversipoa, Homalopoa,* gynodioecious, gynomonecious, and dioecious species, among others). A fourth group, "B" (including *Australopoa*), is separated from group C by a single RS gain in the latter group. *Poa sieberiana* lacks a *Bcl* I RS that is synapomorphic for IVC, yet no more can be said about this RS since not all taxa have been tested. Moreover, it could represent a reversal. Group IV subdivisions were supported by eight RS between groups A and C, and C2 was distinguished from C1 by four RS synapomorphies.

Five of the 11 shortest cladograms occurred within group IVC1. Alternative trees placed *P. bigelovii, P. chamaeclinos, P. piperi, R. rhizomata, P. tracyi,* and *P. hybrida* in differing combinations, as sister taxa to the remainder of C1 or as derived from within the group. Most of the alternative cladograms arose from gaps in the data that result in the first four taxa attaching within C1, or between C1 and IVA. A *Pvu* II RS absence whose polarity is uncertain caused *P. tracyi* and *P. hybrida* to join the tree either above or below the IVC1 node. This RS was present in all other samples of group IVC and A, in *P. eminens* (group I), and in species of two other Pooideae tribes (Soreng, Davis, and Doyle, in press). Because losses are more likely than parallel gains (Debry and Slade, 1985) it seems best to consider this a loss after a single gain, at least within group IVC. This results in the arrangement of group IV taxa seen in Fig. 2 and in Dollo trees. If the presence of this RS is homologous among the taxa, then it must have originated deep in the phylogeny

TABLE 3. Poa *chloroplast DNA insertion/deletion events*

Enzyme No.[a]	Region[b]	Change[c]	Taxa[d]
*Bam*H I			
1	6	1.2 → 1.5 = 0.3	1
2	5	4.65 → 4.8 = 0.15	1
3 = 14	5	4.45 → 4.25 = 0.2	9–11
4 = 13	5	4.45 → 4.35 = 0.1	16
5 = 39, 49	5-4	2.6 → 2.5 = 0.1	9–33
6 = 34	3	3.5 → 3.15 = 0.35	1
7	3	1.73 → 1.74 = 0.01	1, 3, 4, 29, 33
8 = 62	3	1.73 → 1.7 = 0.03	12–14
9 = 61	3-2	1.05 → 1.18 = 0.13	1
10	2	6.7 → 6.65 = 0.05	9–46
11 = 36, 64, 69	7	4.95 → 4.8 = 0.15	34–45, 46?
Bcl I			
12	5	2.05 → 1.97 = 0.08	3
13 = 4	5	2.05 → 1.95 = 0.1	16
14 = 3	5	2.05 → 1.85 = 0.2	9–10
15 = 55	2	0.76 → 0.85 = 0.09	26, 28
Bgl II			
16	7	1.5 → 1.55 = 0.05	3
17	7	3.98 → 3.85 = 0.13	7
18	7	2.9 → 2.65 = 0.25	9–33
19	6	1.52 → 1.55 = 0.03	3
20	5	0.8 → 0.78 = 0.02	46
21	5-4	6.9 → 7.0 = 0.1	4
22	5-4	6.9 → 6.3 = 0.6	2
23 = 79	5-4	6.9 → 6.6 = 0.3	7–8, 19, 24
24 = 50	4	3.1 → 3.05 = 0.05	12–14
25	4	3.1 → 3.2 = 0.1	7
26 = 54	4	3.12 → 3.1 = 0.02	1–2, 9–33, 46
27	4	3.5 → 3.49 = 0.01	1
28	4	1.78 → 1.76 = 0.02	1
29	4	1.78 → 1.79 = 0.01	9–11, 15, 17–18 20–26, 28–33
30	4	1.78 → 1.8 = 0.02	12–14, 16, 19, 27
31	4	1.78 → 1.81 = 0.03	4
32	4	1.23 → 1.20 = 0.03	40–45
33 = 46	4	0.95 → 0.94 = 0.01	9–33
34 = 6	3	3.4 → 3.6 = 0.2	1
35	3-2	16.5 → 17.0 = 0.5	46
36 = 11, 64, 69	2-1	3.4 → 3.25 = 0.15	34–46
37	2-1	2.17 → 2.19 = 0.02	1
38	2-1	2.17 → 2.18 = 0.01	13
Cla I			
39 = 5, 49	5	4.05 → 3.95 = 0.1	9–33
40	4	0.7 → 0.86 = 0.16	15
41 = 72	4	0.65 → 0.56 = 0.1	12–14
42	2-1	3.45 → 3.8 = 0.35	3
*Eco*R I			
43	7	2.6 → 2.57 = 0.3	3, 6, 20
44	6	3.66 → 3.65 = 0.01	3, 6, 9–33
45	6	3.66 → 3.45 = 0.21	1, 2
46 = 33	5	2.35 → 2.33 = 0.02	9–33
47 = 58	5	2.35 → 2.32 = 0.03	34–46
48 = 59?	5	1.55 → 1.59 = 0.04	7
49 = 5, 39	5-4	3.4 → 3.27 = 0.16	9–33
50 = 24	4	4.2 → 4.15 = 0.05	12–14
51	4	2.1 → 2.31 = 0.2	12–29, 31–33
52	4	2.1 → 2.32 = 0.21	1
53	4	2.1 → 2.15 = 0.05	8
54 = 26	4	1.4 → 1.38 = 0.02	7–33, 46
55 = 15	2	4.5 → 4.4 = 0.1	26

TABLE 3. *Continued*

Enzyme No.[a]	Region[b]	Change[c]	Taxa[d]
56	2	4.5 → 4.37 = 0.13	34–37, 39
57	2	4.3 → 4.35 = 0.05	34–46
Hind III			
58 = 47	(70–72)	2.05 → 2.00 = 0.05	34–46
59 = 48	(52–61)	9.4 → 9.2 = 0.2	7
60	(37–43)	7.05 → 7.1 = 0.05	4, 35–45, 46?
61 = 9	(32–34)	2.55 → 2.65 = 0.1	1
62 = 8	(29–32)	3.55 → 3.5 = 0.05	12–14
63	(13–16)	3.6 → 4.0 = 0.4	8
64 = 11, 36, 69	(8–13)	5.5 → 5.4 = 0.1	34–45
65	(8–13)	5.5 → 5.6 = 0.1	13
Hpa I			
66	(100–102)	2.7 → 2.6 = 0.1	1
67 = 81	(52–53)	0.9 → 0.84 = 0.06	14
68 = 78	(24–29)	6.4 → 6.6 = 0.2	3–8
Kpn I			
69 = 11, 36, 64	(5–9)	5.0 → 4.9 = 0.1	34–46
70	(0–9)	8.0 → 7.9 = 0.1	3–8, 34–46
71	(0–9)	8.0 → 8.1 = 0.1	9–33
Pst I			
72 = 41	(51–54)	2.7 → 2.6 = 0.1	12, 14
73	(37–45)	9.0 → 9.2 = 0.2	9–45 (73 = 35 taxon 46)
74	(34–37)	3.1 → 2.9 = 0.3	3–46
Pvu II			
75 = 76	(30–35)	5.3 → 5.8 = 0.5	1
Sal I			
76 = 75	(26–34)	6.6 → 6.9 = 0.3	1
77	(23–29)	7.1 → 6.9 = 0.2	40
78 = 68	(23–29)	7.1 → 7.3 = 0.2	3–8
Sma I			
79 = 23	(54–61)	7.2 → 7.1 = 0.1	3–8, 19
Sca I			
80	(39–59)	4.0 → 3.7 = 0.3	11
81 = 67	(39–59)	4.0 → 4.1 = 0.1	14
82	3	1.4 → 1.55 = 0.15	9–33
Xho I			
83	(52–55)	3.7 → 3.65 = 0.05	9–33

[a] Insertion/deletion events are numbered in order by enzyme and probe region, or, where mapped, their inclusive map coordinates are given in parentheses. Equivalent events detected in other digests are indicated by an "=" sign.
[b] Chloroplast regions are coded as in Table 2.
[c] Changes are not polarized, arrows indicate the state in the listed taxa.
[d] Taxa are numbered as in Table 1.

of the subfamily Pooideae and may have been lost as many as ten times.

Group V—Group V includes two parts: "D" (encompassing the *P. bulbosa* accession, and the group *Secundae*), and "E" (encompassing species of the *Abbreviatae, Oreinos, Stenopoa* s.s., and *Tricopoa,* with *Coenopoa* basal). The union of group V, marked by only one RS, was supported by four different I/D events.

DISCUSSION

Interpretation of the cpDNA phylogeny of Poa—In this section I discuss congruence between the cpDNA tree (Fig. 2) and traditional classification of the genus *Poa.* Strict cladistic analysis cannot represent the full evolutionary history of reticulately inherited characters. In *Poa* polyploidy and apomixis (which facilitate introgression and perpetuation of hybrids) are

common and diploid species rare, numerous hybrids occur naturally or have been synthesized (Knobloch, 1968), and reticulate origins of certain species groups have been postulated. Although cladistic analysis can be applied as an exploratory tool in reticulating groups, limited attempts to use it on gross morphological and anatomical characters of *Poa* (Soreng, unpublished data) were confounded by homoplasy, some of which evidently derived from reticulation; thousands of shortest trees were generated that exhibited low consistency (0.3–0.4), and all major nodes collapsed in strict consensus trees. The nature of evolution in *Poa* to some extent is responsible for numerous equally parsimonious and often highly contradictory solutions to formal cladistic analyses based on morphological characteristics. One way to circumvent theoretical and practical limitations to cladistic analysis where reticulation is suspected is to define relationships by applying parsimony analysis to linearly inherited characteristics such as cpDNA RS. Caution is needed in interpretation of results where lineage sorting, hybrid origins, and introgression may have occurred (Palmer et al., 1983; Doyle, Doyle, and Brown, in press), but post-facto search for inconsistencies with other types of data can expose possible hybrids. The present cladistic analysis of cpDNA RS in *Poa* revealed a relatively stable phylogenetic structure with low homoplasy.

One measure of the correspondence of the cpDNA cladogram to a species phylogeny, in the absence of a stable cladogram of *Poa* phylogeny based on other data, is the congruence of RS synapomorphies with the existence of morphologically coherent groups and with previously postulated relationships among groups. Examples of congruence between the cpDNA cladogram and morphological groups occurred for many sets of closely allied taxa: 1) *Poa arachnifera* of North America and *P. iridifolia* and *P. lanigera* of South America (*Dioicopoa* s.s.); 2) *P. confinis, P. cusickii, P. fendleriana, P. piperi,* (North American dioecious species of *Madropoa*); 3) *P. tracyi, P. cuspidata, P. nervosa, P. rhizomata* (North American gynodioecious species, *Nervosae*); 4) *P. bigelovii* and *P. occidentalis* (*Diversipoa*); 5) *P. candamoana* and *P. chamaeclinos* (*Punapoa*); 6) *P. arctica* and *P. pratensis* (*Poa*); 7) *P. secunda* and its putative sexual counterparts *P. napensis* and *P. tenerrima* (*Secundae*); 8) *P. alsodes, P. saltuensis,* and *P. autumnalis* (*Sylvestres*); 9) *P. palustris, P. nemoralis,* and *P. compressa* (*Stenopoa*); 10) *P. keckii* and *P. brachyanthera* (*Abbreviatae*); 11) *P. laxa* (unpublished data) and *P. paludigena* (*Oreinos*).

Additional support for the congruence of the cpDNA phylogeny and morphology comes from the placement of some unique groups in positions consistent with previous hypotheses. 1) *Bellardiochloa* has been placed within *Poa* as a sister group (Pseudofestuca Asch. & Graebner) to the remainder of the genus, or is treated as a distinct genus allied to *Poa* (Edmondson, 1980) or *Festuca* (Tzvelev, 1983). Cladistic analysis of morphological characters (Soreng, unpublished data) placed it outside of *Poa,* and of cpDNA RS (Soreng, Davis, and Doyle, in press) placed it near *Festuca,* separated from *Poa* by *Dactylis* and *Arctagrostis. Bellardiochloa* caryopses have soft to semi-liquid endosperm (as in *Dactylis* and *Arctagrostis* and many genera of the tribe Aveneae) (vs. solid endosperm in *Poa* and most other genera within the Poeae and other tribes of the subfamily) and round backed lemmas (characteristic of most genera in the subfamily) with a short awn (awns being common in the subfamily) (vs. keeled and unawned lemmas in *Poa* [two South American *Poa* have short awns]). 2) *Poa eminens* and four relatives, recently removed to a new genus, *Arctopoa,* were subsequently reunited with *Poa* as subgenus *Arctopoa* (Tzvelev, 1983). These species are postulated to be basally derived within *Poa* (Tzvelev, 1983), and indeed *P. eminens* comes out in a basal group in the cpDNA analysis. 3) *Poa sieberiana* and *P. labillardierei,* species of a distinctive group of tussock grasses endemic to New Zealand and Australia, exhibited a cpDNA RS pattern little differentiated from the generalized group IVC type common in North and South America. *Australopoa* are unlikely to have arisen from hybridization with species from other continents and always formed a clade with the very similar *P. stricta* of North America in cladistic analyses of morphology. The relative ease of crossing between the tussock grass *P. "cespitosa"* (a problematic name, once widely applied to species of this group) from Australia and *P. arachnifera* from North America (Clausen, 1961), as compared to the difficulty of crosses between these and other species tested (Hiesey and Nobs, 1982), supports the inclusion of *Australopoa* in group IVC. 4) *Poa hybrida* is a diploid member of the western Eurasian section *Homalopoa.* It closely resembles certain North American species, particularly *P. occidentalis* (*Diversipoa*) (one of the three known diploids in North America) and *P. tracyi,* with which

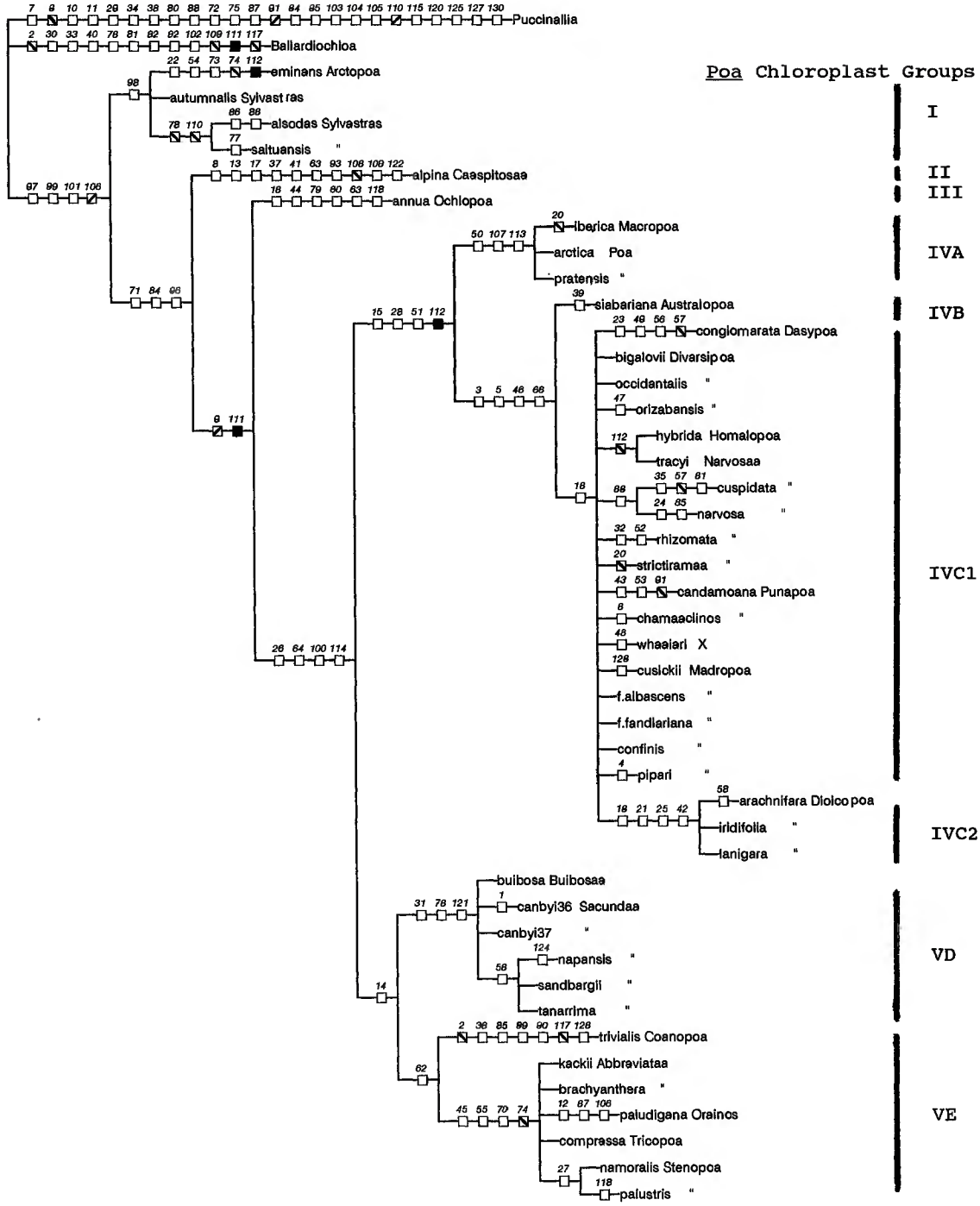

Fig. 2. One of 11 most parsimonious trees of *Poa* chloroplast DNA restriction sites. Of the 11 most parsimonious trees, this tree most closely approximates the strict consensus tree. Excluding autapomorphies, the tree is 87 steps long with a consistency index of 0.84. Numbers over boxes refer to restriction site characters (Fig. 1; Table 2); open boxes represent unique site gains and losses. Putative gain-loss, loss-gain, and parallel loss events (polarized to *Puccinellia*) are indicated within boxes by / for gains and \ for losses; solid boxes represent parallel gains. Group designations specify chloroplast groups discussed in the text, and classification of taxa follows Table 1.

it shares the derived character states of strongly keeled sheaths closed over half their length, exceptionally tall stature, and elongate panicles (Soreng and Hatch, 1983). *Poa hybrida* was the only accession of Eurasian origin found to have a group IVC cpDNA type and it shared a nearly identical RS pattern with *P. tracyi.* (Incidentally, I have found no character support for maintaining *Diversipoa* as distinct from *Homalopoa.*) 5) The dioecious *Madropoa* and *Dioicopoa* s.s. are postulated to be sister groups (Marsh, 1952; Clausen, 1961; Soreng, 1986), and this was not rejected by the cpDNA phylogeny, nor was a putative relationship between these and the North American partially gynodioecious group, *Nervosae* (Soreng, 1986), rejected. 6) *Oreinos, Abbreviatae,* and *Stenopoa,* considered to be closely allied by Edmondson (1980) and Tzvelev (1983) all fall into cpDNA group VE. 7) *Poa trivialis* is considered to represent a distinct section allied to *Stenopoa* and *Abbreviatae* (Tzvelev, 1983), and also falls within group VE. 8) Laterally creeping rhizomes are present in many group IV species but rarely occur outside this group within *Poa.* The rhizomatous habit also coincides with sheaths closed over one-third their length or more within group IV.

Reticulate evolution —Reticulate relationships may have affected the positions of the following five taxa in the cpDNA cladogram (Fig. 2).

1) The *Bulbosae* (including *P. bulbosa*) are usually considered to be closely related to the *Caespitosae* (including *P. alpina*). Edmondson (1978) includes these groups in their own section *Bulbophorum,* and Tzvelev (1983) treats them as two of four subsections within section *Poa.* Although the individual groups are defined by morphological autapomorphies (except for section *Poa*), characters used to infer relationships among them are widespread in the genus (smooth panicle branches, pilose palea nerves, sheaths closed one-fourth to two-thirds their length, anthers 1.2–2.8 mm long). For these and the following reasons I tentatively consider each a separate section. The *Poa alpina* accession had a very distinct cpDNA RS pattern, which suggests that inclusion of the *Caespitosae* in or near section *Poa* should be reevaluated. The position of the *P. bulbosa* accession in Fig. 2 was also far removed from section *Poa,* and it shared three synapomorphic RS with the *Secundae.* Two plausible explanations for this are: *P. bulbosa* (a polyploid apomict) hybridized with the *Secundae* in California (the origin of the accession, and where *P. bulbosa* is adventive), or an unidentified parent (*Puccinellia* has been suggested [Stebbins, personal communication]) hybridized with some relative of *Bulbosae* in the distant past giving rise to the high polyploid, and predominantly apomictic, *Secundae.* To resolve cpDNA relationships in these sections, diploid sexual species of both *Bulbosae* and *Caespitosae,* which occur in Europe and the Mediterranean region, will have to be sampled.

2) The possibility that the *Secundae* are of reticulate origin is postulated because of their possession of a mixture of *Puccinellia* and *Poa*-like characteristics and occurrence of high polyploidy and extensive apomixis (Stebbins, 1950, p. 404). Explicit support for this hypothesis comes from the combination of characters thought to be ancestral within *Poa,* or reticulately inherited in the *Secundae* (rounded lemmas, a crown of callus hair, and prominently papilliate epidermal cells in some species [Soreng, unpublished data]), coupled with a derived cpDNA type characteristic of species with advanced *Poa* morphology.

3) *Poa compressa* is a polyploid apomict clearly allied on morphological grounds to section *Stenopoa,* in which it is placed as a subsection by Tzvelev (1983). It, however, has one unique and one rare character within group V and section *Stenopoa*—strongly creeping rhizomes and highly compressed culm nodes. I suggest that these characters might be derived from hybridization between *P. rhemannii* (Asch. & Graebner) Woloszcz., a diploid *Stenopoa* species with compressed culms (as a chloroplast donor), and *P. pratensis,* a group IV species with similarly strong creeping rhizomes.

4) The placement of *P. iberica* (section *Macropoa*), in group IVA with section *Poa,* requires additional confirmation. *Macropoa* is usually aligned near section *Homalopoa* (group IVC1). The accession used in this study might be of hybrid origin with *P. pratensis,* which forms fertile hybrids with *P. longifolia* Trin. also of section *Macropoa* (Vandijk and Winkelhorst, 1982).

5) *Poa wheeleri,* a high polyploid apomict occurring widely in western North America, is usually submerged within *P. nervosa* as a variety. If the fairly narrow endemic *P. nervosa* s.s. was one of *P. wheeleri*'s parents, some other species (I have suggested *P. cusickii,* and one I/D found was shared between *P. wheeleri* and one of the *P. cusickii* accessions) may have been the chloroplast donor. *Poa nervosa* and *P. cuspidata,* two sexual gynodioecious species disjunct between temperate deciduous forests of eastern and western North America, shared a RS loss. *Poa wheeleri* could have received its

chloroplast from *P. nervosa,* but its origin would then predate the vicariance of *P. nervosa* and *P. cuspidata.* Alternatively, *P. wheeleri* could have derived its different chloroplast through hybridization, introgression, or lineage sorting.

The examples of possible reticulation events cited above suggest caution when interpreting the cpDNA phylogeny in *Poa.* Stebbins may have been right in suggesting *Poa* might represent one huge polyploid complex, yet such complexes often contain structure. Further testing will be required to confirm putative reticulation events. On the other hand, the presence of clearly definable cpDNA groups that correspond to morphologically defined groups and sections agrees with the prediction that there were and are independent lines of evolution in *Poa,* and that the cpDNA cladogram can be used, cautiously, and along with other data, for developing a classification of the genus.

Biogeography —The cpDNA cladogram, coupled with other information, can generate hypotheses of origin and radiation of sectional groups. The center of diversity of *Poa* in number of sections or groups, diversity within groups, and range of cpDNA types, is clearly Eurasia. This agrees with chorological studies that demonstrate Eurasia to be the center of generic and species diversity within the inclusive subfamily Pooideae and tribe Poeae (Hartley, 1961, 1973; Cross, 1980). Of the 11 sections in which diploids are known, seven are centered in, or restricted to, western Eurasia. Of the 19 groups of species studied for RS, nine are restricted to Eurasia, or their primary centers of diversity are in Eurasia with few taxa indigenous to North America. The latter taxa have circumpolar distributions (e.g., *P. alpina, P. arctica, P. eminens, P. laxa* (Oreinos), *P. nemoralis* s.l. (including *P. glauca* Vahl), *P. pratensis*), and all except *P. eminens* and *P. laxa* are known apomicts. The *Abbreviatae* are centered in Berringia where there are several diploid species. The sister group relationship of section *Poa* and cpDNA group IVC, which is supported by morphological similarity and cladistic character analysis, suggests a vicariance event, because section *Poa* has its center of species richness in northeastern Asia and Berringia, whereas group IVC is principally New World.

The cpDNA connection between *Homalopoa,* a group centered in Europe with several diploid species (the accession studied is a diploid collected from Greece) and group IVC is more difficult to explain. The morphology of the group is, as discussed above, strikingly similar to that of some North American species. It may represent a primary link to North America or secondary radiation back to Eurasia. The latter hypothesis seems plausible in view of the fact that RS patterns of *P. tracyi* and *P. hybrida* are nearly identical.

The distribution of the remaining subgeneric groups is predominantly New World or Antarctic. Only the *Sylvestres, Nervosae,* and possibly *Madropoa* can be considered to have cpDNA types principally or entirely confined to North America. *Dioicopoa* s.s., *Dasypoa,* and the gynomonecious *Punapoa* of group IVC are principally South American, the *Secundae* occur in both North and South America, and *Australopoa* are endemic to New Zealand and Australia.

The *Sylvestres,* so far as is known, are endemic to the temperate deciduous forests of eastern North America. They appear to be basal in the cpDNA tree and could represent a group derived from a time when eastern North America was in close floristic contact with the temperate flora of Europe. However, Old World counterparts of the *Sylvestres* have not been identified.

The *Secundae* are a small group in which I include *P. curtifolia* Scribner, *P. napensis, P. secunda* s.l., *P. stenantha* Trin., *P. tenerrima,* and *P. unilateralis* Scribner. Several forms of the facultative apomict, *P. secunda* s.l., are disjunct to South America. Because the *Secundae* cpDNA type is relatively advanced and related to the *Stenopoa* group VE type (principally Palearctic), and the sexual members of the group and species diversity occur in North America, I postulate that they arose here and secondarily spread to South America.

Group IVC species contain the most widespread cpDNA type in North America. This type is clearly derived within the genus and presumably marks the major radiation event among New World *Poa.* By extrapolation to related species I estimate that this type is present in about 50 (ca. 50%) North American and 90 (ca. 90%) South American species. Within group IVC, only *Dioicopoa* s.s., and possibly *Dasypoa,* are well marked by cpDNA RS advancement.

Dioicopoa s.s. includes some 40 species, of which only one occurs north of the equator. The cpDNA type present in the three representatives sampled evidently is derived from the group IVC type. *Poa arachnifera* is endemic to the southern Great Plains where it displays 6*n,* 7*n,* 8*n,* and 12*n* ploidy levels. As there was a polychotomous rather than a dichotomous relationship between this species and two South American representatives, and it is morpho-

logically nearly indistinguishable from two other lower polyploid (4*n*) species from South America, it seems probable that *P. arachnifera*'s occurrence in North America represents an amphitropical dispersal north as is suggested by Marsh (1952) rather than a relic distribution of a stem or sister species involved in the radiation of *Dioicopoa.*

One last geographical connection supports a floristic link between New World species of group IV and those of Australia/New Zealand. *Poa sieberiana* and *P. labillardierei* are representatives of the widespread cytotype in New Zealand (Hair, 1968). If the group IVC cpDNA type is endemic to the New World and most closely related to the circumpolar section *Poa,* then it is reasonable to postulate that the major radiation of *Poa* in the Holoantarctic Floristic Kingdom stemmed from dispersal from North America to South America to the Neozeylandic Region (as is suggested by Takhtajan, 1986) rather than from Asia. The presence of dioecy and rhizomes in some New Zealand species, morphologically close to *Dioicopoa* s.s., also supports this connection, as do crossing relationships (discussed above). (Evidently there are Asian *Poa* connections as well [e.g., between *Poa drummondiana Nees* of Australia and *P. tuberifera* Faurie ex Hack. of far East Asia], but they are minor constituents of the genus in Australia.) *Poa sieberiana* and *P. labillardierei* retain a RS that was lost among the remainder of group IVC species tested. This RS may mark an alternative path of radiation, possibly from Southeast Asia, and raises the possibility that group IV *Poa* arrived in the New World from the antarctic region. However, because not all group IVC species have been screened for this single RS, support for speculation is minimal.

Chloroplast DNA RS patterns demonstrate that dispersal events from North to South America, in different groups of *Poa,* occurred at least twice; once by group V species, and one to several times by group IV species. That amphi-neotropical disjunctions have occurred more frequently than this is indicated by the number of disjunct species or species pairs: (group V) 1) *P. glauca* Vahl (*Stenopoa*), 2) *P. secunda* (three forms), and 3) *P. stenantha* Trin. (*Secundae*); (group IV), 4) *P. arachnifera–P. lanuginosa* Poir. (*Dioicopoa*), 5) *P. douglasii* Nees–*P. cummingii* Nees (*Madropoa*). 6) *Poa acinaciphylla* Desv. (= *P. villaroelei* Phil.) (*Andinae or Punapoa?*), and 7) *P. chamaeclinos* Pilger (*Punapoa*), and 8) *P, conglomerata–P. scaberula* Hook. f. (*Dasypoa*), represent South American group IVC species that occur in North America only on Mexican volcanoes. Thus, the cpDNA cladogram yields a conservative estimate of the number of such dispersal events.

Reflections on the mode of cpDNA radiation in Poa — The limited amount of variation in cpDNA RS in group IVC1 contrasts with the high degree of morphological and physiological differentiation in this group. Breeding system variation is extensive, including self-compatible and self-incompatible hermaphroditism, gynomoecism, gynodioecism, and dioecism. Longevity ranges from annual to long-lived perennial. The species have adapted to many habitats (e.g., temperate rain, coniferous and deciduous forests, riparian habitats, arid-steppe, Mediterranean coastal sand-dunes, warm-temperate grasslands, margins of warm deserts, and temperate and tropical alpine habitats). Laterally creeping rhizomes range from highly developed to absent. Sheaths range from closed to the collar to open nearly to the base. Blades range from broad, thin, and flat, to narrow, thick, and involute. Panicles range from open and averaging 20 cm long with several hundred spikelets, to condensed and averaging 1 cm long with fewer than ten spikelets. In fact, nearly all morphological and anatomical character states found to vary in *Poa* vary within this group.

It is worth noting that excluding the Eurasian section *Homalopoa, P. occidentalis* is the only diploid species known in group IVC. Of some 70 species investigated, the majority are tetraploid or have tetraploid races.

There are several plausible explanations for the lack of hierarchical structure among cpDNA types within this diverse group: 1) There are species groups, but morphological and ecological evolution proceeded more rapidly so that few cpDNA RS markers appeared; 2) There are no groups; radiation proceeded rapidly and independently from one common ancestor; 3) There are groups but they were not exposed because closely related species were not sampled, RS were undersampled, or RS sampling was uneven among species; 4) Some equilibrating process is involved: Possibly common chloroplast genomes circulate through extensive hybridization and introgression along with some paternal leakage of chloroplasts (e.g., *Secundae* could have arisen from hybridization between species with an uncommon chloroplast type and a more widespread type, by chance retaining the more common chloroplast type). Such a system may be operating in *Quercus* (Whittemore, unpublished data). In other words, the most common chloroplasts in a reticulating group are the ones most likely to end up in new hybrid taxa that may subsequently replace their predecessors.

Hypotheses supposing a lack of species groups among the samples may be rejected. There is good morphological evidence for derived groups of closely related species. For example: condensed-panicles, thick-involute leaf-blades with hairy upper surfaces, and reduced upper leaf-blades, are all synapomorphies for *Madropoa,* of which *P. confinis, P. cusickii, P. fendleriana* (two subspecies), and *P. piperi* were sampled, yet no RS synapomorphies (and few autapomorphies), were found. The fourth hypothesis, that of reticulation, is rejected as a primary process, in this case on grounds that reticulation does not explain the limited hierarchical variation between cpDNAs derived from diverse species of Europe, North America, South America, and Australia.

Undersampling of RS is always a possibility, yet several cases have been discovered wherein little or no synapomorphic cpDNA RS variation has been detected among morphologically diverse species (e.g., subsets within genera of trees of the Juglandaceae [Smith and Doyle, personal communication] and Arecaceae [Wilson and Clegg, personal communication] and herbs such as *Antennaria* [Michaels, personal communication]). Morphological evidence indicates that the radiation of group IVC1 resulted in distinct groups but little structure among them. Whether, or to what degree, reticulation is responsible in part for the lack of hierarchical structure in the cladistic analysis of cpDNA RS in group IVC requires further investigation.

Cladistic analysis of cpDNA RS demonstrated the presence of significant phylogenetic structure within the genus and agreed fairly well with some traditionally postulated sectional level species groupings, but refuted others. In addition, the study provided information on putative parentage for further analyses of the origin of polyploid and apomictic complexes. Limited sampling of species in cpDNA group I–III (particularly of diploids), and a lack of samples from Southeast Asian groups, allows only tentative conclusions to be drawn about phylogenetic relationships among certain basal species and groups within *Poa.* However, what has been discovered provides a substantial basis for revision of the subgeneric classification of the genus in the New World, and interpretation of character evolution and biogeographic events.

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