Theor Appl Genet (1990) 80:326–332

THEORETICAL AND APPLIED GENETICS

Chloroplast DNA variation in diploid and polyploid species of *Bromus* (Poaceae) subgenera *Festucaria* and *Ceratochloa*

M. Pillay and K. W. Hilu

Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg/VA 24061, USA

Received January 20, 1990; Accepted April 3, 1990
Communicated by P. L. Pfahler

Summary. Chloroplast DNA (cpDNA) restriction endonuclease patterns are used to examine phylogenetic relationships between *Bromus* subgenera *Festucaria* and *Ceratochloa*. *Festucaria* is considered monophyletic based on the L genome, while *Ceratochloa* encompasses two species complexes: the *B. catharticus* complex, which evolved by combining three different genomes, and the *B. carinatus* complex, which is thought to have originated from hybridization between polyploid species of *B. catharticus* and diploid members of *Festucaria*. All species of subgenus *Ceratochloa* (hexaploids and octoploids) were identical in chloroplast DNA sequences. Similarly, polyploid species of subgenus *Festucaria*, except for *B. auleticus*, were identical in cpDNA sequences. In contrast, diploid species of subgenus *Festucaria* showed various degrees of nucleotide sequence divergence. Species of subgenus *Ceratochloa* appeared monophyletic and phylogenetically closely related to the diploid *B. anomalus* and *B. auleticus* of subgenus *Festucaria*. The remaining diploid and polyploid species of subgenus *Festucaria* appeared in a distinct grouping. The study suggests that the *B. catharticus* complex must have been the maternal parent in the proposed hybrid origin of *B. carinatus* complex. Although there is no direct evidence for the paternal parent of the latter complex, the cpDNA study shows the complex to be phylogenetically very related to the diploid *B. anomalus* of subgenus *Festucaria*.

Key words: Chloroplast DNA – Phylogeny – *Bromus* – Poaceae – Grasses

Introduction

Bromus L. (bromegrasses, Poaceae) is a genus of about 100 diploid and polyploid species that includes important forage, range, and weedy species (Gould and Shaw 1983). Evolution in the genus is based on polyploidy and hybridization within and/or between the different subgenera, which has given rise to species with complex genomic constitutions (Stebbins 1981). This proposed pattern of evolution has generated questions regarding the phylogenetic relatedness of the different sections of the genus. Stebbins (1956, 1981) presented one of the more comprehensive treatments of *Bromus*. He divided the genus into seven distantly related subgenera (*Festucaria, Ceratochloa, Neobromus, Stenobromus, Bromus, Nevskiella,* and *Boissiera*), and proposed an evolutionary scheme based primarily on information from gross morphology, chromosome size, and analyses of meiotic chromosome pairing in a few interspecific hybrids. The effectiveness of this information in resolving phylogenies is sometimes limited by factors such as parallel and convergent evolution in morphology, high degree of phenotypic plasticity (Clayton 1981), and difficulties of hybridizing species from the different subgenera. Other limitations include problems associated with interpreting chromosome pairing in hybrids, particularly those of polyploid origin, due to asynaptic or desynaptic mutations or rediploidization of polyploids (Dewey 1982; Jackson 1984; Kaul and Murthy 1985).

In this study, chloroplast DNA (cpDNA) variation is used to assess the phylogenetic relationships between polyploid species of subgenus *Ceratochloa* and species of subgenus *Festucaria*. Chloroplast DNA, being evolutionarily highly conserved, has been very useful in inferring phylogenetic relationships in polyploid complexes (Tsunewaki and Ogihara 1983; Perl-Treves and Galun 1985; Ichikawa et al. 1986; Kishima et al. 1987; Hilu 1988; Hosaka and Hanneman 1988; Hosaka et al. 1988; Soltis and Soltis 1989; Lumerat et al. 1989). Chloroplast DNA is mostly maternally inherited in flowering plants

(Kirk and Tilney-Basset 1978; Sears 1980) and, consequently, the phylogenies resulting from this study will be discussed in that frame.

Proposed genomic relationship between Festucaria and Ceratochloa

Subgenera *Festucaria* Grenier & Godron (*Pnigma* and *Bromopsis* of Dumortier) and *Ceratochloa* Beauv. contain approximately 60 and 16 species, respectively (Smith 1970). In each subgenus there are valuable pasture species, such as *B. inermis, B. carinatus,* and *B. catharticus* (Stebbins and Tobgy 1944). Subgenus *Festucaria* includes both diploid and polyploid species. The diploid (2n = 14) and tetraploid species are distributed primarily in the Americas, while species with higher ploidy levels such as hexaploids, octoploids, and decaploids have a Eurasian distribution (Armstrong 1981). Subgenus *Ceratochloa,* on the other hand, consists entirely of higher polyploid species. The *Ceratochloa* species fall into two morphologically distinct complexes: the *B. catharticus* complex, which are hexaploids (2n = 42) endemic to South America, and the *B. carinatus* complex, which are octoploids found mainly in North America (Stebbins 1981).

Stebbins (1956) and Armstrong (1987) proposed a monophyletic origin for the *Festucaria* species, all of which contain different numbers of the L genome. The two complexes of subgenus *Ceratochloa* are assumed to combine three ($A_1B_1B_2$) or four ($A_1B_1B_2L$) genomes (Stebbins 1981). The hexaploid *B. catharticus* complex is considered to have evolved from presently extinct diploid and tetraploid species that contributed the A_1, B_1, and B_2 genomes. The *B. carinatus* complex is thought to have originated from hybridization between members of the hexaploid *B. catharticus* complex and diploid species of *Festucaria* (Stebbins and Tobgy 1944; Stebbins 1947, 1981). Therefore, the *B. carinatus* complex is assumed to contain the A_1, B_1, B_2 and L genomes. Stebbins and Tobgy (1944) suggested that different species of the diploid *Festucaria* have entered into the ancestry of the *B. carinatus* complex, favoring a partly polyphyletic origin for the group.

The genomic relationships proposed by Stebbins (1956, 1981) also imply that subgenera *Festucaria* and *Ceratochloa* have different ancestry. Stebbins (1981) indicated that the relationships between the two subgenera are obscure and their chromosomes so dissimilar that a recent common origin is difficult to imagine.

Materials and methods

Plant material

The two subgenera were represented in this study by 15 diploid and polyploid species. The species used, their chromosome numbers, and sources of seed material are listed in Table 1. Plants were grown from seeds under greenhouse conditions. In most cases, seeds were sown in 15-cm pots and leaves were repeatedly harvested when the plants were 3–4 weeks old. In the case of *B. inermis* and *B. carinatus,* large amounts of seeds were sown in 28 × 53 cm flats. All plants were destarched in the dark for 1 or 2 days before leaf harvests. Leaf material was either used immediately for cpDNA extraction, or freeze-dried in liquid nitrogen for storage in a –70 °C freezer.

cpDNA extraction, restriction enzyme digestion, and electrophoresis

Chloroplast DNA was isolated using the method of Kemble (1987) with minor modifications. For each extraction, 25 g of leaf material was ground in a mortar with a pestle using liquid

Table 1. List of *Bromus* species, their chromosome numbers, and sources of seed material used in this study

Species	Chromosome no.	Accession no.	Source of material
Subgenus *Festucaria*			
B. porteri (Coult) Nash	14[a]	74–34	K. Armstrong, Canada
B. ciliatus L.	14[a]	387908	P. I. Station, Pullman
B. anomalus Rupr.	14[a]	232200	P. I. Station, Pullman
B. auleticus Trin. ex Nees	42[a]	162779	P. I. Station, Pullman
B. inermis Leyss.	56[a]		
B. pumpellianus Scribn.	56	70–140	K. Armstrong, Canada
B. biebersteinii Roem. et Schult.	70	372614	P. I. Station, Pullman
Subgenus *Ceratochloa*			
B. catharticus Vahl.	42[a]		Daehnfeldt Inc, Oregon
B. coloratus Steud.	42	202696	P. I. Station, Pullman
B. stamineus Desv.	42	251107	P. I. Station, Pullman
B. brevis	42	384465	P. I. Station, Pullman
B. valdivianus Phil.	42	368864	P. I. Station, Pullman
B. carinatus H. & A.	56[a]		
B. sitchensis Trin.	56	202534	P. I. Station, Pullman
B. breviaristatus Buckl.	56	392355	P. I. Station, Pullman

[a] Chromosomes numbers confirmed by squashing technique

Table 2. Chloroplast DNA data matrix for 15 species of *Bromus* showing proportion of shared fragments (*F* values; upper right) and estimates of cpDNA nucleotide sequence divergence ($\sigma \times 100$; lower left) following Nei and Li (1979)

	1	2	3	4	5	6	7	8
1. *B. porteri*	–	0.912	0.902	0.927	0.966	0.966	0.966	0.901
2. *B. anomalus*	0.516	–	0.859	0.984	0.931	ß.931	0.931	0.988
3. *B. ciliatus*	0.576	0.852	–	0.866	0.921	0.921	0.921	0.863
4. *B. auleticus*	0.422	0.086	0.802	–	0.946	0.946	0.946	0.972
5. *B. pumpellianus*	0.192	0.397	0.458	0.306	–	1.000	1.000	0.920
6. *B. biebersteinii*	0.192	0.397	0.458	0.306	0.000	–	1.000	0.920
7. *B. inermis*	0.192	0.397	0.458	0.306	0.000	0.000	–	0.920
8. *B. catharticus* and *B. carinatus* complexus[a]	0.585	0.064	0.824	0.152	0.465	0.465	0.465	–

[a] Species belonging to these two complexes are listed in Table 1

nitrogen. The powder was then resuspended in 400–500 ml of the isolation buffer. This method of grinding resulted in little or no shear of the cpDNA in comparison with the grinding of leaves by homogenizing with a Waring blender.

Chloroplast DNA was digested with the restriction enzymes AvaII, BamHI, BglI, EcoRI, HindIII, KpnI, and SalI according to the supplier's instructions. The DNA fragment digests were resolved on 0.5%–1% agarose gels, depending on the number of fragments generated by the enzyme. Gels were stained with ethidium bromide and photographed under ultraviolet light. Lambda DNA-HindIII fragments (Bethesda Research Laboratories) were used as size standards.

Analysis of data

The cpDNA restriction endonuclease patterns of the various species were scored for fragment length differences. The data obtained from using the different enzymes were pooled, and a genetic distance matrix was then computed using Nei and Li (1979) Eqs. (21) and (20), which calculate the F ($F=2n_{xy}/[n_x+n_y]$) and Sigma values ($F \approx P^4/3-2P$), respectively. These algorithms are put forth to estimate genetic distances from fragment length differences instead of restriction sites. The genetic distance matrix (Sigma values) were then analyzed by the Unweighted Pair-Group Method (UPGMA) using the NT-SYSpc package of computer programs developed by Rohlf (Version 1.50, 1989). The genetic distance matrix was also analyzed by the Fitch and Margoliash (1967) cladistic method for genetic distances using the PHYLIP 3.1 computer program developed by J. Felsenstein. In the latter analysis, the Jumble option was used for entering the species in a random order.

Results

The enzymes EcoRI, BamHI, HindIII, AvaI, KpnI, BglI, and SalI revealed mutational events that produced differences in restriction fragment sizes between at least two species. A representative restriction fragment pattern of the species is presented in Fig. 1 for the endonuclease HindIII. The restriction endonucleases BglI, KpnI, and SalI generated an average of 11 fragments, while the others produced an average of 25 fragments per species. Of the total 2,093 fragments produced by all the restriction enzymes, 1,760 fragments (84%) were shared by all 15 species of *Bromus*. The percent shared fragments (*F* values) and genetic distances calculated from these data are presented in Table 2.

When the genetic distance matrix was subjected to UPGMA analysis, three distinct clusters of species were apparent (Fig. 2a). One cluster included the diploid *B. porteri*, octaploids *B. inermis* and *B. pumpellianus*, and decaploid *B. biebersteinii*, all members of subgenus *Festu-*

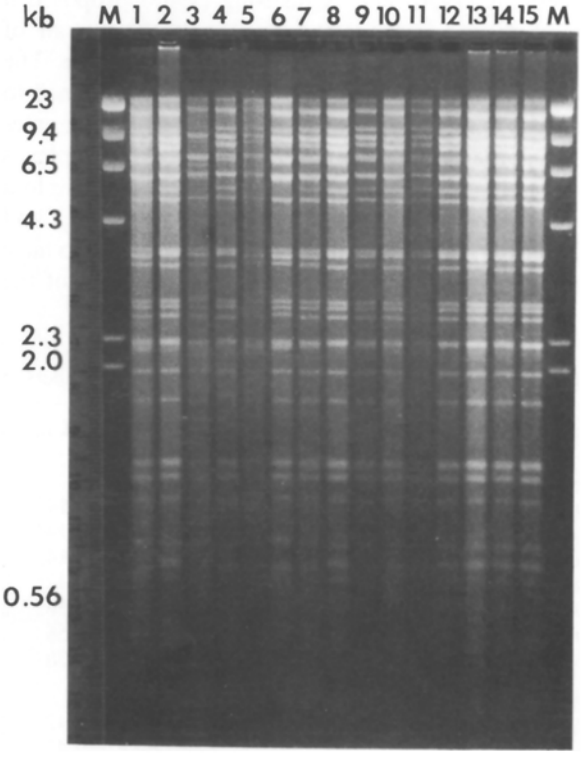

Fig. 1. HindIII endonuclease restriction fragment patterns of cpDNAs from the 15 species of *Bromus*: *B. porteri* (*1*), *B. anomalus* (*2*), *B. ciliatus* (*3*), *B. auleticus* (*4*), *B. pumpellianus* (*5*), *B. biebersteinii* (*6*), *B. inermis* (*7*), *B. carinatus* (*8*), *B. breviaristatus* (*9*), *B. sitchensis* (*10*), *B. catharticus* (*11*), *B. brevis* (*12*), *B. valdivianus* (*13*), *B. coloratus* (*14*), and *B. stamineus* (*15*). *M* = Lambda DNA – HindIII restriction fragments used as size markers

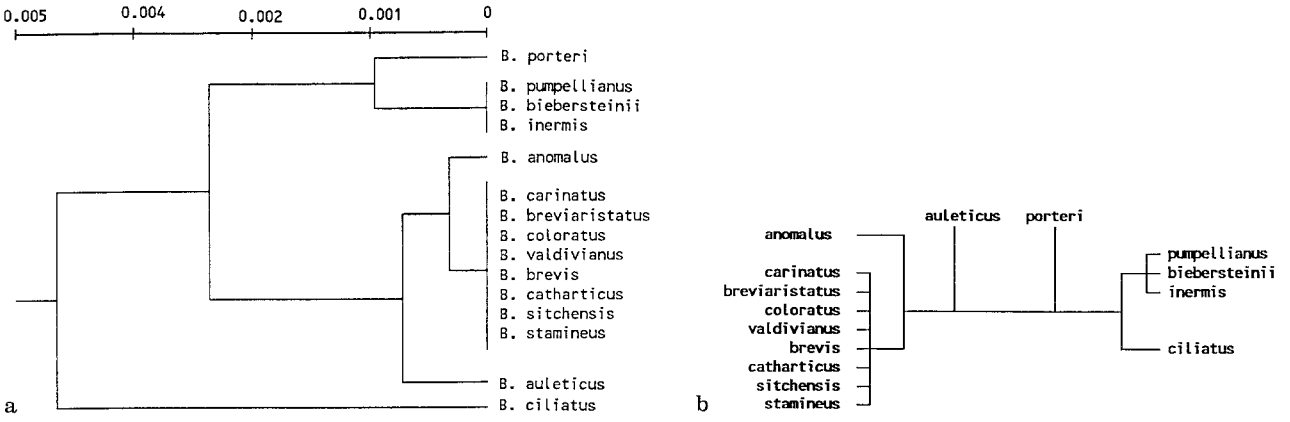

Fig. 2 a and b. Phenogram and cladogram of the 15 species of *Bromus* based on a genetic distance matrix calculated with Nei and Li's (1979) genetic distance measures. **a** Phenogram based on the Unweighted Pair-Group method (UPGMA); **b** cladogram based on the genetic distance method of Fitch and Margoliash (1967)

caria. The second cluster included the diploid *B. anomalus* and hexaploid *B. auleticus* of subgenus *Festucaria*, as well as all members of subgenus *Ceratochloa* (the hexaploids *B. catharticus, B. valdivianus, B. stamineus, B. coloratus*, and *B. brevis* of the *B. catharticus* complex, and the octaploids *B. carinatus, B. sitchensis*, and *B. breviaristatus* of *B. carinatus* complex). The third one included the North American *B. ciliatus*, a diploid species of subgenus *Festucaria*. The cophenetic correlation between the grouping generated by the UPGMA and the genetic matrices was very high ($r=0.953$), indicating a very low level of distortion.

The network obtained from using Fitch and Margoliash's (1967) distance method is shown in Fig. 2b. Species of subgenus *Ceratochloa* and *B. anomalus* (subgenus *Festucaria*) appeared monophyletic, and phylogenetically closely related to *B. auleticus*. *Bromus pumpellianus, B. inermis*, and *B. biebersteinii*, which were identical in the cpDNA nucleotide sequences, appeared in the same clade with *B. ciliatus;* all species are members of subgenus *Festucaria*. *Bromus porteri* was more distant from the *B. pumpellianus* clade, which is incongruent with the UPGMA results (Fig. 2a).

Discussion

One of the most striking features of this study is the lack of cpDNA sequence divergence among most polyploid species within each subgenus. All members of subgenus *Ceratochloa* (both hexaploid and octaploids) were identical in cpDNA fragment patterns. Similarly, polyploid species of subgenus *Festucaria*, with the exception of *B. auleticus*, revealed no cpDNA nucleotide sequence differences. The cpDNA of these species was digested with restriction endonucleases such as EcoRI, BamH1, and HindIII that produce a large number of fragments, and thus are useful in revealing mutational events between closely related taxa. In contrast, the restriction endonucleases did reveal various numbers of mutational events in the diploid species.

The cpDNA results could imply that the two groups of polyploid species with identical restriction endonuclease site sequences had monophyletic plastome origins, and that they have not accumulated resolvable mutational events since their divergence, because of the slow rate of evolution of the chloroplast genome. Stebbins (1981) suggested that (1) the archaic species of *Bromus* originated in Eurasia in the Miocene, (2) the polyploid species of *Ceratochloa* differentiated in Eurasia and their hexaploids spread to North America during the Pliocene, and (3) the octoploid species of *Ceratochloa* evolved in North America during the Pleistocene and Recent. Therefore, the lack of mutational differences among cpDNAs of the polyploids is possible if one considers a maternal monophyletic origin, Pliocene differentiation, and the estimated synonomous rate of nucleotide substitution for cpDNA of 1.1 nucleotide substitution/site/10^9 year (Zurawski et al. 1984), and the 0.7% sample of the cpDNA genomes surveyed in this study. The rates of synonomous nucleotide substitutions tend to be higher than the rates of nonsynonomous substitution (Clegg et al. 1984; Birky 1988), and the overall rate of cpDNA nucleotide substitution was estimated to be $0.3-0.8 \times 10^{-9}$ year (Birky 1988). Therefore, if those estimates are used, then the monophyletic plastome origin and the Pliocene differentiation becomes even more likely.

The literature on cpDNA restriction endonuclease analysis does not include any studies involving complex polyploid species such as in *Bromus*. However, it appears that diploid species generally show more variability in their restriction fragment patterns than their related polyploids. This situation was particularly true in *Triticum* and *Aegilops* (Bowman et al. 1983), and for

groups of species in *Cucumis* (Perl-Treves and Galun 1985), *Beta* (Kishima et al. 1987), and *Dactylis* (Lumaret et al. 1989). In the case of *Triticum* and *Aegilops*, Tsunewaki (1989) has indicated that plasmon diversity decreases with the increase of ploidy level. While this pattern of the plastome is true in this study, the situation in *Bromus* subgenus *Ceratochloa* is an extreme in that nine species representing the full geographic distribution of the subgenus (both North and South America) were identical with respect to restriction sites of seven enzymes.

Members of subgenus *Ceratochloa* grouped together by virtue of their identical restriction site sequences (Fig. 2). However, species of subgenus *Festucaria* appeared in three clusters and clades (Fig. 2a and b). The cpDNA results suggest that the Eurasian species of subgenus *Festucaria* (*B. inermis, B. pumpellianus,* and *B. biebersteinii*) are related to the North American diploids *B. porteri* (UPGMA, Fig. 2a) and *B. ciliatus* [Fitch and Margoliash (1967) method, Fig. 2b]. On the other hand, the North American diploid species *B. anomalus* and the South American hexaploid *B. auleticus* show high affinities to members of *Ceratochloa*. The disparity between the UPGMA and the Fitch and Margoliash methods of analysis is in terms of the position of *B. ciliatus* and is not a significant one. *Bromus ciliatus* had higher *F* values and smaller genetic distance with the *B. pumpellianus* group and with *B. porteri* than with the other species of *Festucaria* and subgenus *Ceratochloa* (Table 2).

The presence of the species of *Festucaria* in three groups raises the question of the cohesiveness of the subgenus. Dumortier (1823, cf. Wagnon 1952) divided the species of what is now called *Festucaria* into sections *Bromopsis* and *Pnigma*. Armstrong (1983) indicated that the smaller chromosome species would fall under *Pnigma*, while the larger chromosome species would fall under *Bromopsis*. This cpDNA study does not reflect that division, since species with large chromosomes (*B. porteri, B. ciliatus, B. auleticus,* and *B. anomalus*) fall into two different lineages (Fig. 2). Armstrong (1983) also indicated that the results from cross compatibilities do not provide sufficient information for the subdivision of the section. A further study of the remaining subgenera of *Bromus* is needed before a decision should be made on the integrity of *Festucaria* as a subgenus.

In *Festucaria*, octoploids *Bromus inermis* and *B. pumpellianus* are considered subspecies, since they intercross easily and their F_1 progeny are fertile (Elliot 1949a; b; Nielsen et al. 1962). However, hybrids between their tetraploid cytotypes show some degree of disruption in chromosome pairing, but fertility was restored to 50% in amphiploids (Armstrong 1982, 1987). Based on the lower fertility at the tetraploid level, Armstrong (1987) suggested the treatment of *B. inermis* and *B. pumpellianus* as two different species. He indicated that the evolution of *B. inermis* and *B. pumpellianus* has probably involved autoploidy, alloploidy, and F_1 hybridization and genetic introgression between different populations and ploidy levels of the two species (Armstrong 1985). The cpDNA study emphasizes the strong genetic affinity between these two species. The cpDNA study also suggests that the decaploid *B. biebersteinii* shares at least a maternal genomic ancestor with *B. inermis* and *B. pumpellianus*.

The lack of cpDNA sequence variation in subgenus *Ceratochloa* presents a case where evolution in the chloroplast genome lags behind changes in the nuclear genome. Various degrees of intersterility barriers have evolved between species of the two complexes and among species of each complex. The wide distribution of the *B. carinatus* complex from Alaska to Mexico has given rise to morphologically intermediate populations. Hybrids formed between these populations range from fertile to completely sterile, depending on the morphological similarity of the parent populations (Stebbins 1981). The reproductive isolation was more pronounced in the *B. catharticus* complex, where interspecific hybrids are either completely sterile as to seed set or weakly fertile (Hall 1955; Stebbins 1947). The two complexes are maintained as distinct species since they are morphologically quite distinct and differ in the presence of the L. genome in the *B. carinatus* group (Stebbins 1981). The disparity in the evolution of the chloroplast and nuclear genomes in these high polyploid complexes is in contrast with the coevolutionary changes between these genomes reported by Kung et al. (1982) and Timothy et al. (1979) in tobacco and maize.

The identical cpDNA nucleotide sequences of the two complexes of *Ceratochloa* indicate that the *B. catharticus* complex may have been the maternal parent in the proposed hybrid origin of the *B. carinatus* complex. Although there is no direct evidence for the paternal parent of the latter complex, the cpDNA study shows the complex to be phylogenetically related to the diploid *B. anomalus* of subgenus *Festucaria* (Fig. 2). This relationship provides indirect evidence that a diploid species of *Festucaria* might have been involved in the parentage of the *B. carinatus* complex.

The cpDNA divergence values between subgenera *Festucaria* and *Ceratochloa* are within the range of those found in other studies at the intrageneric level. The genetic distances between species in this study ranged from 0.0000 to 0.0082. The *p* values calculated for various monocot and dicot species belonging to the same genus ranged from 0.0017 to 0.0156 (Palmer and Zamir 1982; Clegg et al. 1984; Sytsma and Gottlieb 1986; Doebley et al. 1987). However, the genetic distance value of 0.0006 found between *B. anomalus* and the *B. catharticus-carinatus* complexes is very low for species belonging to taxa that are considered to be two separate genera

(Tsvelev 1976) or even two subgenera (Stebbins 1981). These genetic distance values do not support Stebbins' statement that the relationships between subgenera *Festucaria* and *Ceratochloa* are obscure, and their chromosomes so dissimilar that a recent common origin is difficult to imagine.

Acknowledgements. We are grateful to Dr. K. C. Armstrong of Ottawa Research Station, Canada, for his valuable comments on this manuscript and for providing seed material of some of the species. We thank the Plant Introduction Station of the U.S. Department of Agriculture, Pullman/WA, for providing most of the other seed material. This study has been supported in part by a grant from Sigma Xi to M. Pillay.

References

Armstrong KC (1982) Hybrids between the tetraploids *Bromus inermis* and *Bromus pumpellianus*. Can J Bot 60:476–482

Armstrong KC (1987) Chromosome evolution in *Bromus*. In: Tcuchiya T, Gupta TK (eds) Chromosome engineering in plant genetics and breeding. Elsevier, Amsterdam (in press)

Armstrong KC (1985) Chromosome pairing in octoploid F_1 and octoploid amphiploid hybrids between *Bromus inermis* and *B. pumpellianus*. Can J Genet Cytol 27:538–541

Armstrong KC (1983) The relationship between some Eurasian and American species of *Bromus* section Pnigma as determined by the karyotypes of some F_1 hybrids. Can J Bot 61:700–707

Armstrong KC (1981) The evolution of *Bromus inermis* and related species of *Bromus* sect. *Pnigma*. Bot Jahrb Syst Pflanzengesch Pflanzengeogr 102:427–443

Birky CW (1988) Evolution and variation in plant chloroplast and mitochondrial genomes. In: Gottlieb LD, Jain SK (eds) Plant evolutionary biology. Chapman and Hall, London, pp 23–53

Bowman CM, Bonnard G, Dyer TA (1983) Chloroplast DNA variation between species of *Triticum* and *Aegilops*. Location of the variation on the chloroplast genome and its relevance to the inheritance and classification of the cytoplasm. Theor Appl Genet 65:247–262

Clayton WD (1981) Evolution and distribution of grasses. Ann Mo Bot Gard 68:5–14

Clegg MT, Rawson JRY, Thomas K (1984) Chloroplast DNA variation in pearl millet and related species. Genetics 106:449–461

Dewey DR (1982) Genomic and phylogenetic relationships among North American perennial Triticeae. In: Estes JR, Tyrl RJ, Brunken JN (eds) Grasses and grasslands. University of Oklahoma Press, Norman/OK, pp 55–58

Doebley J, Renfroe W, Blanton A (1987) Restriction site variation in the *Zea* chloroplast genome. Genetics 117:139–147

Elliot FC (1949a) The cytology and fertility relations of *Bromus inermis* and some of its relatives. Agron J 41:298–303

Elliot FG (1949b) *Bromus inermis* and *B. pumpellianus* in North America. Evolution 3:142–149

Fitch WM, Margoliash E (1967) Construction of phylogenetic trees. Science 155:279–284

Gould FW, Shaw RB (1983) Grass systematics, 2nd edn. McGraw-Hill, New York

Hall BM (1955) Genetic analysis of interspecific hybrids in the genus *Bromus*, section *Ceratochloa*. Genetics 40:175–192

Hilu KW (1988) Identification of the "A" genome of finger millet using chloroplast DNA. Genetics 118:163–167

Hosaka K, Hanneman RE Jr (1988) The origin of the cultivated tetraploid potato based on chloroplast DNA. Theor Appl Genet 76:172–176

Hosaka K, Zoeten GA de, Hanneman RE Jr (1988) Cultivated potato chloroplast DNA differs from the wild type by one deletion – Evidence and implications. Theor Appl Genet 75:741–745

Ichikawa H, Hirai A, Katayama T (1986) Genetic analysis of *Oryza* species by molecular markers for chloroplast genomes. Theor Appl Genet 72:353–358

Jackson RC (1984) Chromosome pairing in species and hybrids. In: Grant WF (ed) Plant biosystematics. Academic Press, Toronto, pp 67–86

Kaul MLH, Murthy TGK (1985) Mutant genes affecting higher plant meiosis. Theor Appl Genet 70:449–466

Kemble RJ (1987) A rapid, single leaf, nucleic acid assay for determining the cytoplasmic organelle complement of rapeseed and related *Brassica* species. Theor Appl Genet 73:364–370

Kirk JTO, Tilney-Bassett RAE (1978) The plastids. Elsevier/North-Holland, Amsterdam

Kishima Y, Mikami T, Hirai A, Sugiura M, Kinoshita T (1987) *Beta* chloroplast genomes: analysis of fraction 1 protein and chloroplast DNA variation. Theor Appl Genet 73:330–336

Kung SD, Zhu YS, Shen GF (1982) *Nicotiana* chloroplast genome. 3. Chloroplast DNA evolution. Theor Appl Genet 61:73–79

Lumaret R, Bowman CM, Dyer TA (1989) Autopolyploidy in *Dactylis glomerata* L.: further evidence from studies of chloroplast DNA variation. Theor Appl Genet 78:393–399

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Nielsen EL, Drolsom PN, Jalal SM (1962) Analysis of F_2 progeny from *Bromus* species hybrids. Crop Sci 2:459–462

Palmer JD, Zamir D (1982) Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon*. Proc Natl Acad Sci USA 79:5006–5010

Perl-Treves R, Galun E (1985) The *Cucumis* plastome: physical map, intrageneric variation, and phylogenetic relationships. Theor Appl Genet 71:417–429

Rohlf FJ (1989) Numerical taxonomy and the multivariate analysis system. Exeter Publishing, New York

Sears BB (1980) Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. Plasmid 4:233–255

Smith P (1970) Taxonomy and nomenclature of the bromegrass (*Bromus* L. s.l.). Notes R Bot Gard Edinburgh 30:361–376

Soltis DE, Soltis PS (1989) Allopolyploid speciation in *Tragopogon:* insights from chloroplast DNA. Am J Bot 76:1119–1124

Stebbins GL (1947) The origin of the complex of *Bromus carinatus* and its phytogeographic implications. Contrib Gray Herb Harv Univ 165:42–55

Stebbins GL (1956) Cytogenetics and evolution in the grass family. Am J Bot 43:890–905

Stebbins GL (1981) Chromosomes and evolution in the genus *Bromus* (Gramineae). Bot Jahrb Syst Pflanzengesch Pflanzengeogr 102:359–379

Stebbins GL, Tobgy HH (1944) The cytogenetics of hybrids in *Bromus*. 1. Hybrids within the section "Ceratochloa". Am J Bot 31:1–11

Sytsma KJ, Gottlieb LD (1986) Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* section *Peripetasma* (Onagraceae). Evolution 40:1248–1261

Timothy DH, Levings III CS, Pring DR, Conde MF, Kermicle JL (1979) Organelle DNA variation and systematic relationships in the genus *Zea:* Teosinte. Proc Natl Acad Sci USA 76:4220–4224

Tsunewaki K (1989) Plasmon diversity in *Triticum* and *Aegilops* and its implication in wheat evolution. Genome 31:143–154

Tsunewaki K, Ogihara Y (1983) The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops* species: II. On the origin of polyploid wheat cytoplasms as suggested by chloroplast restriction fragments. Genetics 104:155–171

Tsvelev NN (1976) Poaceae URSS Tribe 4 Bromeae Dum. USSR Academy of Science Press, Leningrad

Wagnon KH (1952) A revision of the genus *Bromus,* section Bromopsis, of North America. Brittonia 7:415–480

Zurawski G, Clegg MT, Brown AHD (1984) The nature of nucleotide sequence divergence between barley and maize chloroplast DNA. Genetics 106:735–749