RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS OF CHLOROPLAST DNA, MITOCHONDRIAL DNA, AND RIBOSOMAL DNA IN TURFGRASSES

by

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(ABSTRACT)

Restriction fragment length polymorphisms (RFLPs) of chloroplast DNA (cpDNA), mitochondrial DNA (mtDNA), and ribosomal DNA (rDNA), were used to estimate and compare the extent of diversity among the organellar and nuclear genomes, and to infer species relationships. Eight cultivated turfgrass species and subspecies were used in this study including: *Festuca rubra* spp, *rubra*, *F. rubra* spp *commutata* Gaud., *F. rubra* spp *trichophylla* Gaud., *F. longifolia* Thuill., *F. ovina* L, *F. arundinacea* Shreb, *Lolium perenne* L., and *Poa pratensis* L. Genomic DNA from tissue samples of 208 cultivars representing the eight turfgrasses was digested with each of four restriction enzymes: *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, and probed with a set of ten barley cpDNA clones, nineteen wheat mtDNA clones, and one wheat rDNA clone.

The degree, type, and distribution of diversity detected within and between these species and subspecies were compared by RFLP analysis. Relative phenotypic diversity in the cytoplasmic and nuclear genomes was evaluated using Shannon's information statistic. Genetic similarities used for computing species relationships were based on the proportion of shared RFLP fragments.

Substantial inter- and intra-specific nuclear and cytoplasmic DNA variation was detected with RFLP markers in the eight turfgrass species and subspecies. Comparison of phenotypic diversity estimates indicates that, in general, the highest

level of variation was detected by rDNA, followed by mtDNA, and the lowest was by cpDNA. The high variability in rDNA indicates that rDNA in these species evolves at a faster rate than both cpDNA and mtDNA, and cpDNA evolves at a slower rate than mtDNA. Species relationships derived based on the data of the three genomes indicate that the five fine fescues are clustered in the same group in agreement with the traditional classification. Relationships among the eight turfgrasses based on mtDNA data are in accordance with those from cpDNA. Furthermore, results from these analyses indicate that *F. arundinacea* and *L. perenne* are closely related to each other, and *P. pratensis* has a rather low degree of relationship to any of the turfgrasses studied.

This is the first study where the data from three genomes have simultaneously been used to address genetic variation and species relationships in plants. The results of this study further indicate that RFLPs of cpDNA, mtDNA and rDNA are useful markers for species and variety identification, and as criteria in germplasm collection as well as in the elimination of duplicates accessions in germplasm banks.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Turfgrasses, an important source of natural vegetation, serve a functional purpose by controlling wind and water erosion of soil, eliminating dust and mud problems, helping to remove air pollutants, and providing an ideal surface for sports fields. They have and are being developed to conserve our soil and water resources, to protect our environment, and to provide recreation and enjoyment for all people. Today, turfgrasses are most widely used in the highly developed industrial regions of the world such as North America, England, Europe, New Zealand, Japan, and Australia. The turfgrass industry is one of the major agricultural industries, and will become more and more important for the future environment. Thus, large areas of turfgrasses will replace other plant species such as trees and bushes, especially around cities, due to their function and low maintenance requirements.

Festucoideae subfamily contains three turfgrass tribes, and one of the most important tribes is *Festuceae*, which includes three major turfgrass genera (*Festuca* L., *Lolium* L., and *Poa* L.). Eight turfgrasses within these three genera include *Festuca rubra* ssp. *rubra, F. rubra* ssp. *commutata* Gaud., *F. rubra* ssp. *trichophylla* Gaud., *F. longifolia* Thuill., *F. ovina* L., *F. arundinacea* Shreb., *Lolium perenne* L., and *Poa pratensis* L.. They are cool-season grasses widely distributed throughout the temperate and subarctic climates such as the cool humid, cool subhumid, and cool semiarid climates. They are the most extensively utilized as turfgrasses in the United States and Europe.

Plant germplasm is a natural resource and genetic diversity is the raw material for plant breeding. For developing effective breeding strategies, breeders must be aware of the genetic diversity available in the species of interest. To develop effective programs for germplasm evaluation, conservation and breeding, and to understand species phylogenetic relationships and evolutionary processes, it is important to measure the level of variability of nuclear as well as chloroplast and

mitochondrial genomes.

Current advances in electrophoresis technology and biotechnology, including molecular genetics, have provided useful tools and made possible the study of genetic variation at the molecular level in higher plants. This variability can be measured by molecular markers such as proteins, isozymes, and DNA. Biochemical markers such as seed storage proteins and isozymes are useful in examining species variability. For example, seed storage proteins have been used to clarify relationships within the primary gene pool for beans, lentils, chickpea, pigeonpea, watermelon, and peanut (Gepts, 1990). Isozyme studies have been applied to banana, barley, millet, maize, rice, and tomato (Doebley, 1989). In addition, isozyme variation has been commonly used for studies of natural diversity in wild relatives (e.g., *Hordeum spontaneum*, Jana and Pietrzak, 1988). These biochemical methods have been valuable in differentiating and identifying some turfgrass cultivars. Electrophoresis of proteins has been used to identify *Agrostis* cultivars (Wilkinson and Beard 1972, Clark *et al.* 1989). Analysis based on isozyme systems have proven useful as a diagnostic tool in differentiating several turfgrass species such as *Stenotaphrum* sp. (Green *et al.* 1981), *Festuca* spp. (Villamil *et al.* 1982), *Poa* sp. (Wu *et al.* 1984), *Lolium* (Ferguson and Grabe 1986), and *Cynodon* spp. (Vermeulen *et al* 1991).

Although biochemical markers have proven to be very useful, they do not provide a complete picture of genetic diversity. This is because they are based on molecular characters which are frequently influenced by environmental or physiological factors. In particular, only a relatively small proportion of genes present in the genome are actually transcribed and translated into proteins at any given time, and environmental conditions, physiological age, and the overall state of the plant can affect the factors that control expression of such proteins.

Recently, methods to analyze the genome at the DNA level have been developed. Molecular genetic techniques have produced new information on the amount of genetic variation present in species of interest. This has proven especially

useful in genetic studies, and has already shown tremendous potential for analyzing problems in plant genetics, evolution, and phylogenetic relationships (Saghai Maroof *et al.* 1984; Palmer *et al.* 1985a,b; Palmer *et al.* 1988; Clegg 1989; Harris and Ingram 1991; Soltis *et al.* 1989a,b; Kim *et al.* 1992). Restriction fragment length polymorphisms (RFLPs), as DNA markers, provide a rapid approach for obtaining estimates of ribosomal DNA (rDNA), chloroplast DNA (cpDNA), and mitochondrial DNA (mtDNA) variability in population samples. This is particularly true for research on the extent and distribution of variation within a species or between related species where DNA markers are considered to fulfill many of the criteria required for such studies. Since RFLP genetic markers are screened directly at the DNA level, an unlimited number of genetic loci can be detected easily. In addition, they show high levels of polymorphism, provide markers throughout the genome, and their expression is unaffected by the environment (Clegg, 1989). Other biochemical markers, such as seed proteins or isozymes, do not fulfill these criteria to the same extent, although they can be assayed easily in plant populations. RFLP markers, therefore, provide a reliable index of plant genotypes.

Estimates of cpDNA, mtDNA, and rDNA variation based on RFLPs have been reported for numerous angiosperms, particularly in major food crops. Despite the economic importance of turfgrasses in developed countries, they have received much less attention than other crops. Studies of genetic diversity, phylogenetic relationships, evolution, and evaluation of germplasm in turfgrass species at the DNA level have not been sufficiently investigated. Ohmura *et al.* (1993) reported cpDNA and rDNA variation in *Festuca, Lolium*, and *Poa* genera but the data were based on only one or two accessions for each species. Limited information on the extent and distribution of cpDNA and rDNA variation is available. Little is known about mtDNA variation in turfgrasses. Therefore, a study of genetic variability in turfgrass species at the molecular level is proposed.

This study represents the first large-scale sampling in cultivated turfgrass

species using RFLPs as genetic markers to: 1) estimate the level, type, and distribution of cytoplasmic diversity in chloroplast and mitochondrial genomes both within and between species of turfgrass; 2) investigate the amount and patterns of variability of rDNA; 3) compare the extent of polymorphisms detected using cpDNA, mtDNA, and rDNA as molecular markers; and 4) infer species and subspecies relationships based upon the data of cpDNA, mtDNA, and rDNA.

Results from these fundamental genetic studies are expected to provide some informative data which can be used to address questions on the evolution and phylogenetic relationships which can not be answered by conventional methods. In addition, this study is expected to provide meaningful information to aid in germplasm evaluation and conservation, and in developing breeding programs.

This dissertation is subdivided into three major chapters entitled:

1. Chloroplast DNA Polymorphism in Three Turfgrass Genera: *Festuca* L., *Poa* L., and *Lolium* L.

2. Mitochondrial DNA Polymorphism in Three Turfgrass Genera: *Festuca* L., *Lolium* L., and *Poa* L.

3. Ribosomal DNA Polymorphism in Three Turfgrass Genera: *Festuca* L., *Lolium* L., and *Poa* L..

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Chapter I

Introduction

The improvement of any plant is dependent upon the genetic variability existing in the available germplasm. Molecular markers are becoming increasingly useful in measuring the genetic variability in crop plants. The use of chloroplast DNA (cpDNA) markers in genetic studies has already shown tremendous potential for analyzing problems in plant genetics, origin, evolution, and phylogenetic relationships (Palmer *et al.* 1985a; Palmer 1985b,c; Palmer *et al.* 1988; Clegg 1989; Harris and Ingram 1991; Soltis *et al* 1989a,b; Wolf *et al.* 1990; Kim *et al.* 1992). One of the many important contributions of restriction fragment length polymorphism (RFLP) analysis is its wide application in analysis of genetic variation in natural populations and for exploration of the evolutionary relationships among plant taxa (Saghai Maroof *et al.* 1984; Song *et al.* 1988). Genetic diversity in corn and rice germplasm has been studied extensively using isozyme and DNA data (Doebley et al. 1983; Goodman and Stuber 1983; Clegg 1989; Messmer *et al.* 1991, 1992; Dudley *et al.* 1991; Melchinger *et al.* 1991; Smith *et al.* 1992; Zhang *et al.* 1992). In addition, some geneticists suggest that the chloroplast genome plays a role in cytoplasmic male sterility (Chen *et al.* 1990). Therefore, an assessment of the extent of cytoplasmic genetic diversity is important in evaluating germplasm resources for broadening the genetic base of crop plants. This information is useful in germplasm evaluation and conservation and in plant breeding programs.

All plants contain three separate genomes: nuclear, chloroplast, and mitochondrial. Although relatively few genes are located in the chloroplast genome, they are a genetically and functionally important part of a plant's genetic information and play an indispensable role in the key processes of photosynthesis. The chloroplast genome is small and relatively constant in size with many copies per cell.

This genome is highly conserved in the evolution of plants and is maternally inherited in the majority of plant species (Kirk and Tilney-Bassett 1978). These unique features of cpDNA have made its analysis relatively simple, rapid, and inexpensive. Use of cpDNA as a marker to measure genetic diversity has been reported for many plant species including *Lupinus texensis* (Banks and Birky 1985), *Linum* spp. (Coates and Cullis 1987), *Hordeum vulgare* and *H. spontaneum* (Holwerda *et al.* 1986; Neale *et al.* 1988), *Zea* spp. (Doebley *et al.* 1987), *Helianthus bolanderi* and *H. annuus* (Rieseberg *et al.* 1988), *Heuchera micrantha* (Soltis *et al.* 1989a), *Tolmiea menziesii* (Soltis *et al.* 1989b), and *Coreopsis* spp. (Crawford *et al.* 1990). These studies have demonstrated that assaying for cpDNA variation is a reliable and useful tool to study phylogenetic relationships, evolutionary processes, and evaluation of germplasm. More recently, studies have demonstrated that intraspecific cpDNA variation is very common and extensive among different plant species (Soltis *et al.* 1992).

Despite the economic importance of turfgrasses in developed countries, they have received much less attention than other crops in the study of genetic variation at the DNA level. Biochemical methods have been valuable in differentiating and identifying some turfgrass cultivars and only a few studies have been reported for distinguishing turfgrass germplasm based on isozyme techniques. Previous studies based on these techniques have proven useful for genotypic identification of several turfgrass species such as *Agrostis* (Wilkinson and Beard 1972; Clark *et al.* 1989), *Stenotaphrum* spp. (Green *et al.* 1981), *Festuca* (Villamil *et al.* 1982), *Poa pratensis* (Wu *et al.* 1984), *Lolium* (Ferguson and Grabe 1986), and *Cynodon* spp. (Vermeulen *et al.* 1991). Genetic variation, evolution, phylogeny, and evaluation of germplasm in turfgrass species at the DNA level has not been investigated and little is known about intraspecific and interspecific cpDNA variation. Species classification and assumed phylogenetic relationships in turfgrasses are based on variation in morphological and physiological characteristics. Since this variation is dependent on the expression of nuclear genes and interactions of nuclear and cytoplasmic genes

with each other and with the environment, this information does not always represent the true genetic variation. The major objectives of this work were: 1) to estimate the level and types of cytoplasmic diversity in cpDNA both within and between species and subspecies of cultivated turfgrasses, and 2) to elucidate the phylogenetic relationships among these species and subspecies.

Materials and Methods

Plant materials

A total of 208 cultivars representing three major cool-season turfgrass genera were assessed for RFLPs of cpDNA. These genera were: 1) *Festuca* L. which contains two subgenera: the fine fescues and the coarse fescues, the former includes: *Festuca rubra* ssp. *rubra* (here after referred to as *F. rubra*); *F. rubra* ssp. *commutata* (*F. commutata*); *F. rubra* ssp. *trichophylla* (*F. trichophylla*); *F. longifolia*; and *F. ovina*; and the latter includes: *F. arundinacea*, 2) *Poa* L. which contains *Poa pratensis*, and 3) *Lolium* L. which contains *Lolium perenne*. These cultivars were entries in the 1989 National Turfgrass Test and are listed in Table 1. Seeds of each cultivar were planted in field plots (3 x 3 feet for each plot) in April, 1990, at the Turfgrass Research Center at VPI & SU in Blacksburg, Virginia. Ten pounds of nitrogen were applied per acre per year. When the grass was 2.5 inches in height it was mowed. The plots were not irrigated and no insect control practices were carried out. In May, 1991, a bulk sample of 15 grams of fresh leaf tissue from each cultivar (approximately 20 individuals) was randomly collected from each plot (one of the three replications), frozen immediately with dry ice, and then stored at -80 ^{o}C.

Source of cpDNA Clones

A set of ten cpDNA (designated as P1 to P10) clones (probes) from a *Pst* I

Table 1. Turfgrass cultivars and sources of plant material analyzed.

Genus, species and/or subspecies	Cultivar	Sources of seed material
Festuca genus:		
F. rubra ssp *commutata*		
(chewings fescue)	NK 82492	NK lawn & Garden
	Molinda	Van der Have Oregon, Inc.
	Barnica	Barenbrug USA
	Shadow	Turf-seed, Inc.
	Atlanta	Van der Have Oregon, Inc.
	Mary	Van der Have Oregon, Inc.
	Jamestown	Loft's seeds, Co.
	Waldorf	Van der Have Oregon, Inc.
	Epsom	Van der Have Oregon, Inc.
	N-105	Normarc, Inc.
	Raymond	Van der Have Oregon, Inc.
	Estoril	International Seeds
	Wilma	E. F. Burlingham & Son
	BAR FR 9F	Barenbrug USA
	Capitol	Ampac Seed Company
	Enjoy	International Seeds
	Koket	E.F. Burlingham & Sons
F. rubra ssp *rubra*		
(creeping red fescue)	Elanor	Production Service
	Franklin	Van der have Oregon, Inc.
	Sylvester	Van Der Have Oregon, Inc.
	WW RS 130	E.F. Burlingham & Sons
	ZW 42-158	Green Genetics Inter.
	PST-4NI	Pure-Seed, Testing
	Bargena	Barenbrug USA
	Bar FR8RC3	Barenbrug USA
	Cindy	International Seeds
	Boreal	National Turf. Feder. Inc.
	WW RS 138	E.F. Burlingham & Sons
	PST-4C8	Pure-Seed, Testing
	Herald	International Seeds
	Longfellow	International Seeds
	Ensylva	International Seeds
	Jasper	Pickseed West

(Table 1 continued)

Genus, species and/or subspecies	Cultivar	Sources of seed material
F. rubra ssp. *trichophylla* (slender creeping fescue)	Bar FR 9P	Barenbrug USA
	Barlotte	Barenbrug USA
	Barcrown	Barenbrug USA
	FRT-30149	Pure-Seed, Testing
	HF 102	Van der Have Oregon, Inc.
	Marker	International Seeds
	LD 3488	Daehnfeldt
	ZW 42-160	Green Genetics Inter.
	HF 138	Van der Have Oregon, Inc.
F. longifolia (hard fescue)	Serra	Willamette Seed Co.
	Melody	Van der Have Oregon, Inc.
	Attila	KWS Seeds
	Aurora	Turf-Seed, Inc.
	Biljart	Van der Have Oregon, Inc.
	Valda	International seeds
F. ovina (sheep fescue)	MX 86	Jacklin Seed Co.
	Bighorn	Turf-Seed, Inc.
F. arundinacea (tall fescue)	Adventure	Warren'sTurf Nursery
	Trident	Seed Research of Oregon
	Titan	Seed Research of Oregon
	Pick DDF	Pickseed West, Inc.
	Pick 127	Pickseed West, Inc.
	Pick SLD	Zajac Performance Seeds
	PE-7E	Reed Funk - Rutgers
	Hubbard 87	Reed Funk - Rutgers
	Legend	Agway
	Taurus	Turf Merchants, Inc.
	Sundance	Seaboard Seed Co.
	Fatima	Van der Have Oregon, Inc.
	Normarc 25	Reed Funk - Rutgers
	Willamette	Willamette Seed & Grain
	Chieftain	Roberts Seed Co.

(Table 1 continued)

Genus, species and/or subspecies	Cultivar	Sources of seed material
	Thoroughbred	Pickseed West, Inc.
	Pick TF9	Pickseed West, Inc.
	PST-50L	Turf-Seed, Inc.
	PST-5D7	Pure-Seed Testing, Inc.
	Cimmaron	LESCO, Inc.
	Bonanza	Cenex Seed Plant
	PST-5AG	Pure-Seed Testing, Inc.
	PST-5BL	Pure-Seed Testing, Inc.
	PST-5MW	Pure-Seed Testing, Inc.
	Trailblazer	LESCO, Inc.
	Jaguar	Zajac Performance Seeds
	PST-DBC	Pure-Seed Testing, Inc.
	Olympic	Turf-Seed, Inc.
	Jaguar II	Zajac Performance Seeds
	Apache	Turf-Seed, Inc.
	PST-5DM	Pure-Seed Testing, Inc.
	Pick DM	Pickseed West, Inc.
	Pacer	International Seeds, Inc.
	Carefree	International Seeds, Inc.
	Richmond	Jonathan Green, Inc.
	Tip	NPI Seed, Inc.
	KY-31	University of Kentucky
	Bel 86-1	Jack Murray - USDA, ARS
	PST-5EN	Pure-Seed Testing, Inc.
	Finelawn 5GL	Finelawn Research Corp.
	Rebel	Loft's Seed, Inc.
	Tribute	Loft's Seed, Inc.
	Arid	Jacklin Seed Co.
	Wrangler	Jacklin Seed/LESCO, Inc.
	Mesa	Jonathan Green, Inc.
	JB-2	Jacklin Seed Co.
	Falcon	E. F. Burlingham
	Syn GA	O. M. Scott & Sons
	Pick GH6	Pickseed West, Inc.
	PST-5HF	Northrup King Co.
	Monarch	Turf-Seed, Inc.

(Table 1 continued)

Genus, species and/or subspecies	Cultivar	Sources of seed material
	Normarc 99	Normarc, Inc.
	PST-5F2	E. F. Burlingham
Poa genus:		
Poa pratensis		
(Kentucky bluegrass)	Barzan	Mount Emily Seeds, Inc.
	Gnome	Turf Merchants, Inc.
	P-104	Loft's Seed, Inc.
	Ram-1	Jacklin & Loft Seed Inc.
	Compact	Tib Szego Associates
	Joy	Green Seed Company
	Sydsport	E. F. Burlingham & Sons
	Georgetown	Loft's Seed, Inc.
	Somerset	Loft's Seed, Inc.
	Able 1	Warren's Turf Nurs. Inc.
	Bar VB 577	Barenbrug Holland
	Annika	Production Services
	Kenblue	National Turf. Feder. Inc.
	Bristol	O. M. Scott & Sons
	Ba 72-5000	Finelawn Research Corp.
	Bar VB 534	Barenbrug Holland
	NE80-88	Uni of Nebraska-T.Riordan
	America	Pickseed West, Inc.
	Ba 69-82	O. M. Scott & Sons
	Ba 73-540	O. M. Scott & Sons
	Parade	Van der Have Oregon, Inc.
	HV 97	Pure-Seed Test, Van der
	Cheri	Jacklin Seed Co.
	Eclipse	Turf Cultivar Assoc.
	Liberty	Zajac Performance Seeds
	Destiny	Jonanthan Green & Son
	Dawn	LESCO, Inc.
	Merion	Jacklin & Loft' Seed Inc.
	239 (Suffolk)	Loft's Seed, Inc.
	Wabash	Loft's Seed, Inc.
	Ikone	Jacklin Seed Co.
	F-1872	Jacklin Seed Co.

(Table 1 continued)

Genus, species and/or subspecies	Cultivar	Sources of seed material
	Aquila	Northrup King Co.
	K 1-152	Northrup King Co.
	Welcome	Rothwell Seeds
	Rugby	Northrup King Co.
	Trenton	Northrup King Co.
	Midnight	Turf-Seed, Inc.
	Challenger	Turf-Seed, Inc.
	Blacksburg	Turf-Seed, Inc.
	PST-CB 1	Pure-Seed Testing, Inc.
	S.D. Certified	National Turf. Feder. Inc.
	WW Ag 468	E. F. Burlingham & Sons
	Mom PP 2926	National Turf. Feder. Inc.
	Bar VB 55	National Turf. Feder. Inc.
	HV-96	National Turf. Feder. Inc.
	Vantage	National Turf. Feder. Inc.
	Classic	Peterson Seed Company
	Ba 72-492	Roberts Seed Co.
	Ba 73-626	Northrup King Co.
	Amazon	Jacklin Seed Co.
	Julia	LESCO & Jacklin Seed Co.
	WW AG 491	E. F. Burlingham & Sons
Lolium genus: *Lolium perenne* (perennial ryegrass)	Barry	Barenbrug Holland
	Tara	Hubbard Seed & Supply
	Barlp 410	Barenbrug Holland
	Yorktown I	Loft's Seed, Inc.
	Palmer	Loft's Seed, Inc.
	Diplomat	Loft's Seed, Inc.
	Pavo	E. F. Burlingham & Sons
	Caliente	NPI Seed, Inc.
	Aquarius	Fred Ledeboer-KWS Einbeck
	Goalie	Northrup King Co.
	Acrobat	Van der Have Oregon, Inc.
	Brenda	Van der Have Oregon, Inc.
	Derby	International Seeds, Inc.
	Gator	International Seeds, Inc.

(Table 1 continued)

Genus, species and/or subspecies	Cultivar	Sources of seed material
	Patriot	Turf Merchants, Inc.
	Rodeo	Turf Merchants, Inc.
	Allaire	Jonathan Green & Sons
	Pick 300	Pickseed West, Inc.
	Ovation	O. M. Scott & Sons
	SR 4000	Seed Research, Inc.
	SR 4031	Seed Research, Inc.
	Pick 647	Roberts Seed Co.
	Ranger	Van der Have Oregon, Inc.
	ISI-K2	International Seeds, Inc.
	Pennfine	Joe Duich-Penn State
	Psu-222	Joe Duich-Penn State
	Mom Lp 763	Van der Have Oregon, Inc.
	Sheriff	Van der Have Oregon, Inc.
	Birdie II	Turf-Seed, Inc.
	Regency	LESCO, Inc.
	PST-2PM	Zajac Performance Seeds
	PST-2DD	Pure-Seed Testing, Inc.
	PST-250	E. F. Burlingham & Sons
	Vintage-2DF	LESCO, Inc.
	PST-259	Turf-Seed, Inc.
	Pst-M2E	Manhat. Rye Grower Assoc.
	246	Turf-Seed, Inc.
	PST-2HH	Pure-Seed Testing, Inc.
	ISI-851	International Seeds, Inc.
	Manhattan	National Turf. Feder. Inc.
	Repell	Loft's Seed, Inc.
	Del 946	Northrup King Co.
	J 207	Jacklin Seed Co.
	J 208	Jacklin Seed Co.
	Linn	National Turf. Feder. Inc.
	Runaway	Van der Have Oregon, Inc.
	Cowboy	Loft's Seed, Inc.
	Delray	Northrup King Co.
	Pick 600	Pickseed West, Inc.
	Manhattan II	Turf Merchants Turf-Seed
	Omega II	Turf-Seed, Inc.

chloroplast genomic DNA library of barley, kindly provided by Dr. T.H.N. Ellis (John Innes Institute, UK), were used in this study. Two reasons for using cpDNA clones from barley were 1) a chloroplast genomic DNA library of turfgrasses was not available; and 2) they were highly homologous to turfgrass DNA and revealed variation in our preliminary studies with four cultivars randomly chosen from each species and subspecies. Plasmids containing each of the ten barley cpDNA clones were transformed into DH5 *alpha* competent cells (*E. coli* bacteria) and plasmid DNA was extracted according to the procedures described by Sambrook *et al.* (1989). Identification numbers and insert sizes of the ten barley cpDNA probes assigned by Day and Ellis (1985) are given in Fig. 1. These clones cover or mark the whole barley chloroplast genome.

DNA preparation and RFLP assays

DNA Isolation--Total cellular DNA was extracted from freeze-dried leaf tissues of each cultivar following previously established procedures (Saghai Maroof *et al.* 1984). The concentration of DNA samples was measured with a DNA Fluorometer TKO100 (Hoefer scientific instruments, San Francisco) according to the manufacturer's protocols. The DNA samples were immediately used for RFLP analysis or stored at 4 ^{0}C.

Restriction Enzyme Digestion and Electrophoresis--The four restriction enzymes; *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I; used in this investigation were purchased from Bethesda Research Laboratories. A sample of about 1 to 3 μg total cellular DNA was individually digested with each of the four restriction enzymes. Altogether, the RFLP data set included 40 probe/enzyme combinations. Digestion was carried out at 37 ^{0}C for 16 hours. Digested DNA fragments were then size-fractionated by electrophoresis in 0.8% agarose gels with a running buffer [1 M Tris, 0.125 M NaAC (sodium acetate), 0.01 M EDTA (ethylenediamine tetraacetic acid), pH 8.1] at 60 milliamps for 24 hours.

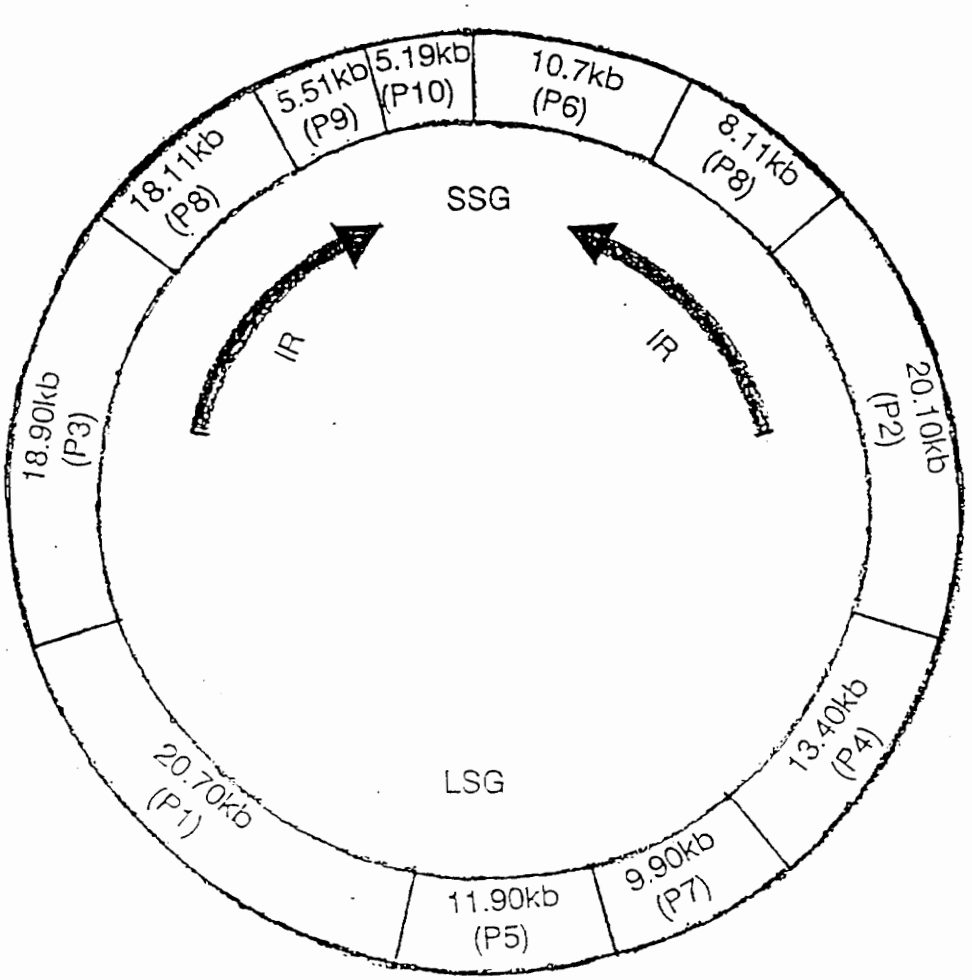

Fig. 1. The map of barley chloroplast genome for Pst I clones (Day and Ellis 1985). LSG=large single copy region; SSG=small single copy region; and IR=inverted repeat sequence.

Southern Blot Hybridization--DNA fragments in the gels were denatured, neutralized, and transferred to nylon membranes following the techniques described by Saghai Maroof *et al.* (1984) with a simple modification. DNA fragments were transferred and blotted onto two nylon membranes (instead of one) which were placed on both sides of each gel. This modification greatly reduced the time and the amount of restriction enzymes required compared to the original method. Pre-hybridization and hybridization were done according to previously established procedures (Neale et al. 1988). Probes were labelled with ^{32}P-dCTP carried out by the random-hexamer primer labeling procedure (Feinberg and Vogelstein 1983) or by nick translation methods (Neale *et al.* 1988). Alternatively, they were labeled with non-radioactive Dig-dUTP using a DNA labeling and detection kit (Boehringer Mannheim) following the manufacturer's instructions. Chloroplast DNA variation was detected through blot hybridization using each of the ten cpDNA probes, one at a time.

Autoradiography--DNA fragments were visualized by autoradiography. Following hybridization, post-hybridizations were carried out by washing the blots twice in 2X SSC (NaCl soduim citrate) and 0.1% SDS (sodium dodecyl sulfate) solution at room temperature for 5 minutes, and once in 0.5X SSC and 0.1% SDS solution at 65 ^{0}C for 10 minutes. After this step, the blots were exposed to Kodak X-ray films with intensifying screens for 8 to 48 hours at -80 ^{0}C.

Comparison of detection of organellar DNA variation based on restriction endonuclease patterns and Southern hybridization

Organellar (cp and mt) DNA polymorphism can be detected in two ways: i) isolation of pure cp or mtDNA, restriction enzyme digestion and direct observation of ethidium-bromide stained agarose gels for data collection, or, alternatively ii) total cellular DNA can be digested by restriction enzymes, Southern blotted and hybridized with cloned cp or mtDNA probes. Data can be collected from X-ray film after

autoradiography. The latter method is preferred for large scale screening and thus was chosen for the present study. One potential problem with this approach is the detection of non-target DNA sequences as a result of cross-hybridization between homologous sequences among chloroplasts, mitochondria, and/or nuclei. For instance mitochondrial and chloroplast DNA sequences have been detected in the nuclear genome (Timmis and Scott 1983; Scott and Timmis 1984), and chloroplast DNA sequences are commonly found in the mitochondrial genomes of higher plants (Stern and Lonsdale 1982; Stern and Palmer 1984; Lonsdale 1985).

Data analysis

To evaluate the degree of cpDNA variation both within and between species and subspecies, Shannon's information statistic (Bowman *et al.* 1971) was calculated for each probe/enzyme combination using the formula $h_s=-\Sigma f_i \ ln \ f_i$, where h_s is the phenotypic diversity value and f_i is the frequency of a given cpDNA phenotype. Only polymorphic probe/enzyme combinations were used for the calculation of phenotypic diversity. Calculation of genetic similarities representing the phylogenetic relationships among these eight turfgrasses were based on the proportion of shared fragments according to Nei and Li (1979). In all cases, the indices of relatedness (F-values) for each pair of these eight turfgrasses were calculated by the formula, F-value$=2 N_{xy}/(N_x + N_y)$, where N represents the total number of fragments hybridizing for species x, N_y represents the total number of fragments hybridizing for species y, and N_{xy} represents the number of fragments shared by each pair of species x and y (Nei and Li 1979). A dendrogram was constructed based on genetic similarities.

Results

Intraspecific cpDNA variation in Festuca genus

cpDNA variation in *F. rubra*--Nine of the 10 cpDNA clones used in this study detected RFLPs in *F. rubra* for at least one of the four restriction enzymes tested (Table 2). Polymorphism was detected for 58% of the 40 probe/enzyme combinations assayed. In many cases, a single clone produced different RFLP patterns with different enzymes. Two of the clones detected polymorphisms with all four enzymes, two with three enzymes, four with two enzymes, and one with one enzyme. Extensive cpDNA variation was detected, and phenotypic diversity values ranged from 0.487 to 1.143 with an average of 0.868. Four phenotypes were detected by Southern hybridization in each of the six probe/enzyme combinations (Table 2). Polymorphism was revealed in the P1/*Eco* RI combination (Fig. 2A, lanes 1 to 4) and four DNA fragment banding patterns were detected. Lanes 1 through 4 all had DNA fragments of 3.2, 2.2, 1.0, 0.94, 0.90, and 0.6 kb. Lane 4 (phenotype d) had an additional fragment of 3.6 kb; this phenotype represents 62% of the total cultivars in this species. In contrast, lane 3 (phenotype c) had a fragment of 4.0 kb. Lane 2 (phenotype b) had additional fragments of 4.0 and 3.6 kb. Lane 1 (phenotype a) had extra fragments of 4.0 and 4.3 kb. When combining the data from all polymorphic probe/enzyme combinations, 11 haplotypes (A through K as shown in the last column of Table 3) were identified among the cpDNAs from 16 *F. rubra* cultivars.

cpDNA variation in *F. commutata*--RFLPs were identified with all ten cpDNA probes for at least one of the four enzymes used. Most probes revealed polymorphisms with more than one enzyme. For example, two of the ten clones demonstrated polymorphism with all four enzymes, two with three enzymes, four with two enzymes, and the remaining two were polymorphic for only one enzyme (Table 4). The highest diversity value was 1.211 and the lowest was 0.227. Up to 60% of the total probe/enzyme combinations were polymorphic in this subspecies. Most

Table 2. Phenotypes, frequencies, and levels of phenotypic diversity detected in cpDNA for 16 *F. rubra* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	Phenotypic diversity
P1/H3*	0.19	0.81			0.487
P1/BH	0.13	0.62	0.19	0.06	1.046
P1/RI	0.13	0.12	0.13	0.62	1.064
P1/XB	0.06	0.69	0.19	0.06	0.910
P3/H3	0.06	0.69	0.25		0.771
P3/BH	0.75	0.25			0.562
P3/RI	0.31	0.69			0.619
P3/XB	0.31	0.69			0.619
P4/BH	0.06	0.69	0.25		0.771
P4/RI	0.06	0.69	0.25		0.771
P4/XB	0.06	0.56	0.38		0.862
P5/BH	0.13	0.62	0.13	0.12	1.080
P5/XB	0.06	0.56	0.38		0.826
P6/RI	0.19	0.56	0.25		0.987
P6/XB	0.13	0.62	0.25		0.907
P7/BH	0.19	0.56	0.25		0.987
P7/RI	0.31	0.69			0.619
P7/XB	0.06	0.56	0.38		0.862
P8/RI	0.19	0.56	0.25		0.987
P8/XB	0.13	0.50	0.31	0.06	1.143
P9/RI	0.19	0.50	0.31		1.025
P9/XB	0.13	0.56	0.25	0.06	1.105
P10/XB	0.13	0.60	0.27		0.924
				Average	0.868

† Only polymorphic probe/enzyme combinations are listed.
* P1-P10 are probes from the barley chloroplast DNA library of Day and Ellis (1985); H3, BH, RI, and XB represent the enzymes *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, respectively.

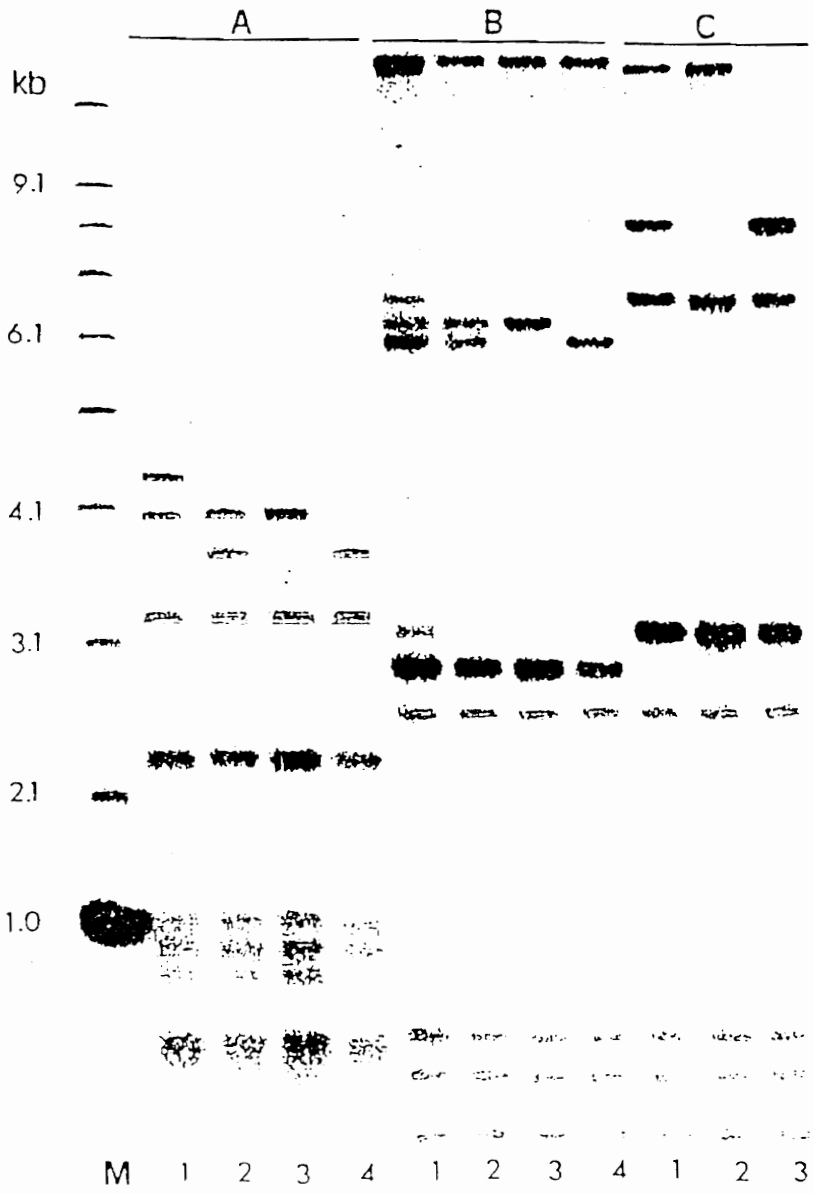

Fig. 2. Autoradiogram of Southern blot showing different cpDNA phenotypes. A: lanes 1-4 showing four phenotypes in *F. rubra* with P1/*Eco* RI; B: lanes 1-4 showing four phenotypes in *F. commutata* with P1/*Xba* I; C: lanes 1-3 showing three phenotypes in *P. pratensis* with P1/*Xba* I. Lane M: 1 kb λ ladder marker.

Table 3. Haplotypes detected among cpDNAs of 16 *F. rubra* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination											Chloroplast haplotype
	P1* H3	P1 BH	P1 XB	P3 H3	P3 BH	P3 XB	P4 XB	P5 BH	P6 RI	P6 XB	P8 XB	P9 XB	
Elanor	a¶	a	a	a	a	a	a	a	a	a	a	a	A
Franklin	b	b	b	b	a	b	b	b	b	b	b	b	B
Sylvester	b	b	b	b	a	b	b	b	b	b	b	b	B
Wwrs130	b	b	b	b	a	b	b	b	b	b	b	b	B
ZW42-158	b	c	c	c	b	a	c	c	c	c	c	c	C
PST-4NI	b	b	b	b	a	b	b	a	b	b	b	b	D
Bargena	a	b	b	b	a	b	c	b	a	b	d	b	E
BaFR8C3	b	b	b	b	a	b	b	b	b	b	b	b	B
Cindy	a	c	c	c	b	a	c	d	c	c	c	c	F
Boreal	b	b	b	b	a	b	c	b	b	b	c	b	G
Wwrs138	b	b	b	b	a	b	b	b	b	b	b	d	H
PST-4C8	b	b	b	b	a	b	b	b	b	b	b	b	B
Herald	b	c	c	c	b	a	c	d	c	c	c	c	I
Longfellow	b	d	d	c	b	a	c	c	c	c	c	c	J
Ensylva	b	a	b	b	a	b	b	b	a	a	a	a	K
Jasper	b	b	b	b	a	b	b	b	b	b	b	b	B

* P1-P9 are probes from the barley chloroplast DNA library of Day and Ellis (1985); H3, BH, XB, and RI represent the enzymes *Hind* III, *Bam* HI, *Xba* I, and *Eco* RI, respectively.

¶ a, b, c, and d represent the phenotypes detected by each corresponding probe/enzyme combination.

Table 4. Phenotypes, frequencies, and levels of phenotypic diversity detected in cpDNA for 17 *F. commutata* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	Phenotypic diversity
P1/H3*	0.59	0.29	0.12		0.924
P1/BH	0.53	0.12	0.35		0.957
P1/RI	0.63	0.31	0.06		0.823
P1/XB	0.06	0.18	0.35	0.41	1.211
P2/XB	0.88	0.12			0.366
P3/H3	0.94	0.06			0.227
P3/BH	0.94	0.06			0.227
P3/RI	0.94	0.06			0.227
P3/XB	0.94	0.06			0.227
P4/BH	0.76	0.24			0.552
P4/RI	0.76	0.24			0.552
P4/XB	0.88	0.12			0.366
P5/BH	0.76	0.12	0.12		0.517
P5/XB	0.88	0.12			0.366
P6/XB	0.94	0.06			0.227
P7/BH	0.88	0.12			0.366
P7/RI	0.88	0.12			0.366
P7/XB	0.88	0.12			0.366
P8/BH	0.88	0.12			0.366
P8/XB	0.76	0.12	0.12		0.517
P9/RI	0.94	0.06			0.227
P9/XB	0.88	0.06	0.06		0.450
P10/BH	0.88	0.12			0.366
P10/XB	0.94	0.06			0.227
				Average	0.459

† Only polymorphic probe/enzyme combinations are listed.
* P1-P10 are probes from the barley chloroplast DNA library of Day and Ellis (1985); H3, BH, RI, and XB represent the enzymes *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, respectively.

polymorphic probe/enzyme combinations revealed two phenotypes, some identified three, and one combination (P1/*Xba* I) detected four phenotypes (Fig. 2B, lanes 1 to 4). Fragments of 14, 2.7, 2.5, 0.7, 0.4, and 0.2 kb were observed in phenotypes shown in lanes 1 to 4. Lane 4 (phenotype d) had an additional fragment of 5.9 kb which is common among 41% of the total cultivars in this species. Lane 3 (phenotype c) had an extra fragment of 6.2 kb. Lane 2 (phenotype b) had two extra fragments of 5.9 and 6.2 kb. In addition to the 5.9 and 6.2 kb fragments, lane 1 (phenotype a) had additional fragments of 6.5 and 3.15 kb. From these results, the chloroplast genomes of 17 *F. commutata* cultivars were classified into 12 types, A through L, as shown in the last column of Table 5.

cpDNA variation in *F. trichophylla*--Polymorphisms of cpDNA in *F. trichophylla* were detected for eight of the ten cpDNA probes with at least one of the four enzymes tested. In total, 15 polymorphic cpDNA probe/enzyme combinations were observed. Two or three phenotypes were detected by most probe/enzyme combinations. Number of observed phenotypes and values of cytoplasmic diversity are shown in Table 6. The range of diversity values was from 0.366 to 1.001 with an average of 0.556. Southern hybridization patterns of *Eco* RI digests of total DNA, probed with the clone P3, detected three different phenotypes among the nine *F. trichophylla* cultivars (Fig. 3, lanes 1 to 3). Fragments of 4.2, 2.4, and 2.2 kb were common for lanes 1 to 3. Lane 2 (phenotype b) had two additional fragments of 3.2 and 1.0 kb which represent most cultivars. Lane 1 (phenotype a) had three extra fragments of 6.4, 3.2, and 1.0 kb. Lane 3 (phenotype c) had an extra fragment of 1.2 kb. Based on the data from four probe/enzyme combinations and as shown in the last column of Table 7 (A through H), the chloroplast genomes of all nine *F. trichophylla* cultivars are different except for cultivars Bar FR 9P and Barcrown.

cpDNA variation in *F. longifolia*--Intraspecific DNA polymorphisms were identified in the chloroplast genome of *F. longifolia*. All ten probes except P8 detected variation within this species. As in *F. trichophylla* and *F. commutata*, most

Table 5. Haplotypes detected among cpDNAs of 17 *F. commutata* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination														Chloroplast haplotype
	P1* H3	P1 BH	P1 RI	P1 XB	P2 XB	P3 XB	P4 BH	P4 RI	P5 BH	P5 XB	P7 XB	P8 XB	P9 RI	P9 XB	
NK 82492	a¶	a	a	a	a	a	a	a	a	a	a	a	a	a	A
Molinda	a	a	a	a	a	a	a	a	a	a	a	a	a	a	A
Barnica	a	a	a	a	a	a	a	a	a	a	a	a	a	a	A
Shadow	a	b	b	b	a	a	a	a	a	a	a	a	a	a	B
Atlanta	b	c	a	c	a	a	a	a	b	a	a	a	a	a	C
Mary	b	c	a	c	a	a	a	a	b	a	a	a	a	a	C
Jamestown	a	a	b	b	a	a	a	a	a	a	a	a	a	a	D
Waldorf	c	b	b	d	b	a	a	b	c	a	a	b	a	b	E
Epsom	c	c	b	c	b	a	b	b	c	a	a	b	a	a	F
N-105	b	c	b	c	a	a	b	a	a	b	b	c	a	a	G
Raymond	a	a	a	a	a	a	a	a	a	a	a	a	a	a	A
storil	b	c	a	c	a	a	b	a	a	a	a	a	a	a	H
Wilma	a	a	c	a	a	a	a	a	a	b	b	c	b	a	I
BAR FR 9F	b	c	b	c	a	a	a	a	a	a	a	a	a	a	J
Capitol	a	a	a	b	a	b	c	a	a	a	c	a	a	c	K
Enjoy	a	a	a	a	a	a	a	b	a	a	a	a	a	a	L
Kolet	a	a	a	a	a	a	a	b	a	a	a	a	a	a	L

* P1-P10 are probes from the barley chloroplast DNA library of Day and Ellis (1985); H3, BH, RI, and XB represent the enzymes *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, respectively.

¶ a, b, c, and d represent the phenotypes detected by each corresponding probe/enzyme combination.

Table 6. Phenotypes, frequencies, and levels of phenotypic diversity detected in cpDNA for nine *F. trichophylla* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	Phenotypic diversity
P1/H3*	0.86	0.14		0.405
P1/BH	0.75	0.25		0.562
P1/RI	0.74	0.13	0.13	0.753
P1/XB	0.86	0.14		0.405
P3/H3	0.78	0.11	0.11	0.680
P3/RI	0.33	0.56	0.11	0.934
P4/BH	0.88	0.12		0.366
P4/RI	0.75	0.13	0.12	0.735
P4/XB	0.88	0.12		0.366
P5/BH	0.43	0.14	0.43	1.001
P6/RI	0.89	0.11		0.347
P7/RI	0.88	0.12		0.366
P9/RI	0.88	0.12		0.366
P9/XB	0.78	0.11	0.11	0.680
P10/XB	0.88	0.12		0.366
			Average	0.556

† Only polymorphic probe/enzyme combinations are listed.

* P1-P10 are probes from the barley chloroplast DNA library of Day and Ellis (1985); H3, BH, RI, and XB represent the enzymes *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, respectively.

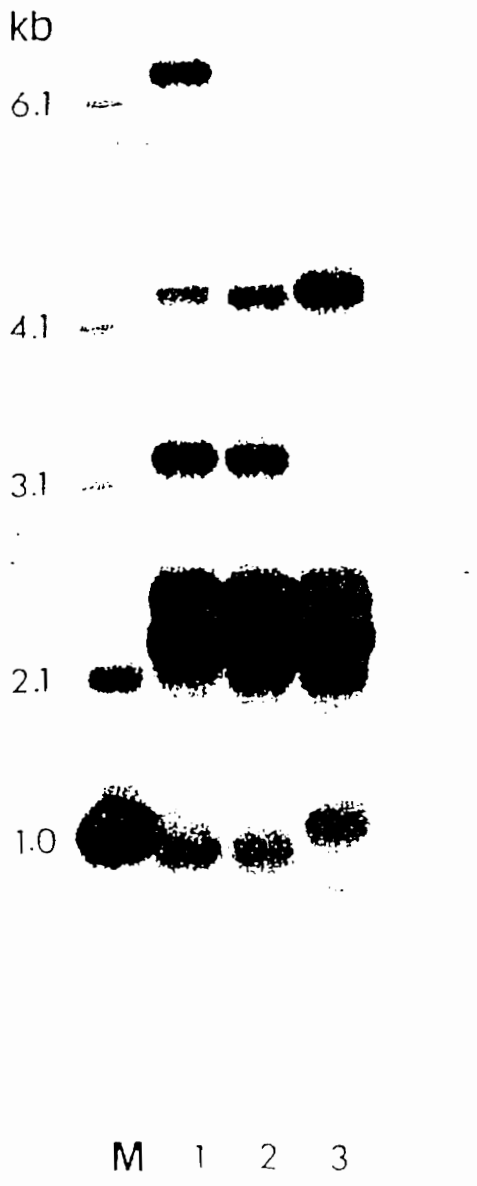

Fig. 3. Autoradiogram of Southern blot showing three cpDNA phenotypes in *F. trichophylla* with P3/*Eco* RI (lanes 1-3). Lane M: 1 kb λ ladder marker.

Table 7. Haplotypes detected among cpDNAs of nine *F. trichophylla* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination							Chloroplast haplotype
	P1* BH	P1 RI	P1 XB	P3 H3	P3 RI	P4 BH	P5 BH	
Bar FR 9P	a¶	a	a	a	a	a	a	A
Barlotte	a	b	a	a	a	a	b	B
Barcrown	a	a	a	a	a	a	a	A
FRT-30149	b	a	b	a	b	a	a	C
HF 102	a	a	a	a	b	a	c	D
Marker	a	a	a	b	a	a	a	E
LD 3488	a	a	a	a	b	a	c	F
ZW 42-160	a	a	a	a	b	a	d	G
HF 138	b	c	a	c	b	b	c	H

* P1-P5 are probes from the barley chloroplast DNA library of Day and Ellis (1985); BH, RI, XB, and H3 represent the enzymes *Bam* HI, *Eco* RI, *Xba* I, and *Hind* III, respectively.

¶ a, b, c, and d represent the phenotypes detected by each corresponding probe/enzyme combination.

polymorphic probe/enzyme combinations detected two phenotypes (Table 8). The P4/*Eco* RI combination revealed four phenotypes which are shown in Fig. 4. Lanes 1 through 4 all had DNA fragments of 3.2, 2.4, 2.2, 0.8, 0.7, 0.5, 0.4, and 0.2 kb. In addition, lane 3 (phenotype c) had a fragment of 1.2 kb. Lane 1 (phenotype a), lane 2 (phenotype b), and lane 4 (phenotype d) all were missing the 1.2 kb fragment, but lanes 1 and 2 had fragments of 2.8 and 2.6 kb, respectively. From Table 9, it can be seen that all six *F. longifolia* cultivars (column 1) can be identified by these polymorphic probe/enzyme combinations (A through F, as shown in the last column).

cpDNA variation in *F. ovina*--Only two cultivars were examined in *F. ovina.* Five of the 40 probe/enzyme combinations were found to be polymorphic. RFLPs were detected with the clones P1, P4, P6, P8, and P9 with one restriction enzyme (*Xba* I or *Eco* RI), and these two cultivars are easily distinguished by Southern hybridization abalysis (Table 10).

cpDNA variation in *F. arundinacea*--Extremely low intraspecific variation was detected in the chloroplast genome of *F. arundinacea.* Only one of the 40 probe/enzyme combinations detected polymorphism. Based on hybridization patterns of the *Xba* I digests, clone P3 revealed two phenotypes among 53 *F. arundinacea* cultivars examined (Fig. 5). Four cultivars were different from the rest. Lane 1 represents the most common type (49 cultivars), and lane 2 is the second type (4 cultivars) which had an extra fragment of approximately 7.1 kb.

Intraspecific cpDNA variation in genus Poa

Fifty-three cultivars of *P. pratensis* were examined with the same set of probes and enzymes as used for the other turfgrasses in *Festuca.* Nine polymorphic probe/enzyme combinations were found among these cultivars. Phenotypes, frequencies, and estimated phenotypic diversity values are summarized in Table 11. Considerably lower levels of diversity were observed within this species as compared to most of the turfgrasses in *Festuca.* Diversity values ranged from 0.098 to 0.539,

Table 8. Phenotypes, frequencies, and levels of phenotypic diversity detected in cpDNA for six *F. longifolia* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	Phenotypic diversity
P1/RI*	0.83	0.17			0.456
P1/XB	0.83	0.17			0.456
P2/BH	0.83	0.17			0.456
P3/H3	0.83	0.17			0.456
P3/RI	0.83	0.17			0.456
P4/BH	0.83	0.17			0.456
P4/RI	0.33	0.17	0.33	0.17	1.334
P5/RI	0.17	0.67	0.16		0.862
P6/XB	0.83	0.17			0.456
P7/RI	0.83	0.17			0.456
P9/XB	0.83	0.17			0.456
P10/XB	0.83	0.17			0.456
				Average	0.563

† Only polymorphic probe/enzyme combinations are Listed.
* P1-P10 are clones from the barley chloroplast DNA library of Ellis and Day (1985); RI, XB, BH, and H3 represent the enzymes *Eco* RI, *Xba* I, *Bam* HI, and *Hind* III, respectively.

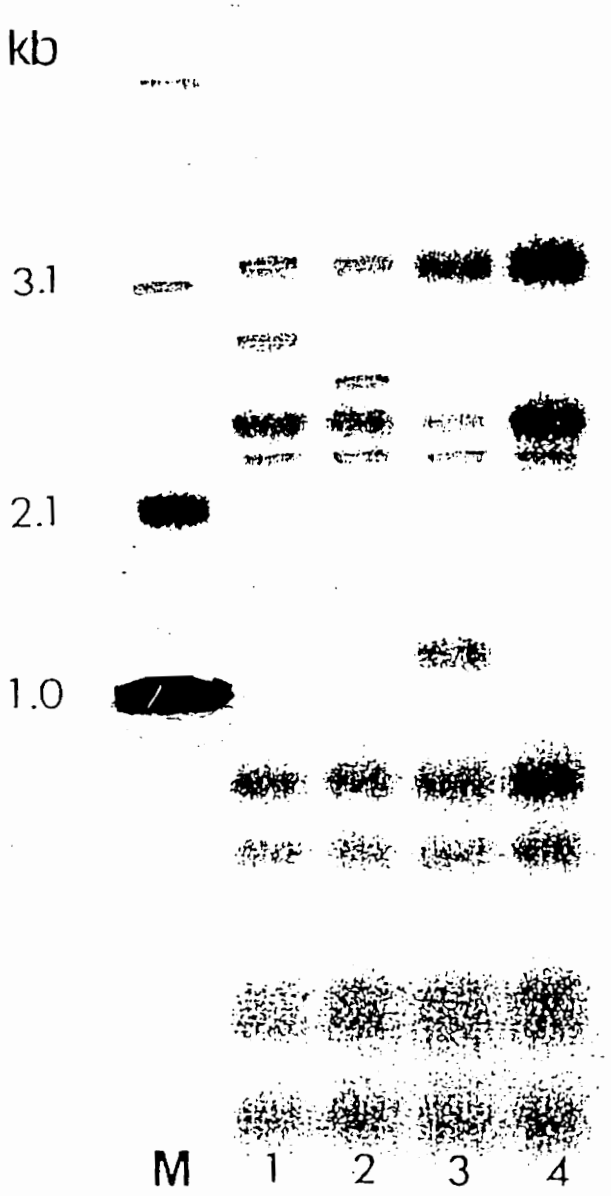

Fig. 4. Autoradiogram of Southern blot showing four cpDNA phenotypes in *F. longifolia* with P4/*Eco* RI (lanes 1-4). Lane M: 1 kb λ ladder marker.

Table 9. Haplotypes detected among cpDNAs of six *F. longifolia* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination		Chloroplast haplotype
	P4* *Eco*R I	P5 *Eco*R I	
Serra	a¶	a	A
Melody	a	b	B
Attila	b	b	C
Aurora	c	c	D
Biljart	c	b	E
Valda	d	b	F

* P4 and P5 are probes from the barley chloroplast DNA library of Day and Ellis (1985).

¶ a, b, and c represent the phenotypes detected by each corresponding probe/enzyme combination.

Table 10. Phenotypes, frequencies, and levels of phenotypic diversity detected in cpDNA for two *F. ovina* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	Phenotypic diversity
P1/XB*	0.50	0.50	0.692
P4/RI	0.50	0.50	0.692
P6/XB	0.50	0.50	0.692
P8/XB	0.50	0.50	0.692
P9/XB	0.50	0.50	0.692
		Average	0.692

† Only polymorphic probe/enzyme combinations are listed.
* P1-P9 are probes from the barley chloroplast DNA library of Day and Ellis (1985); XB and RI represent the enzymes *Xba* I and *Eco* RI, respectively.

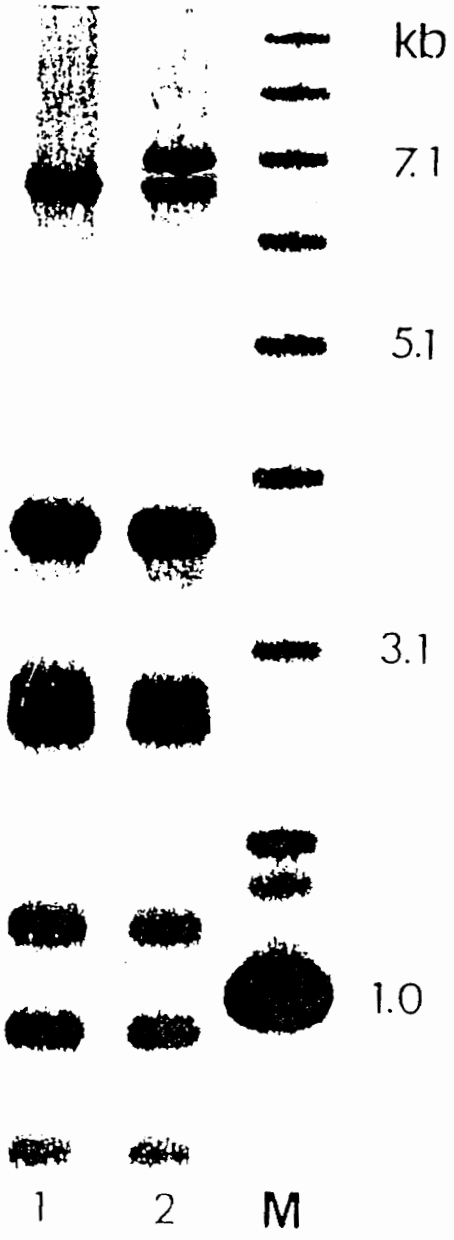

Fig. 5. Autoradiogram of Southern blot showing two cpDNA phenotypes (lanes 1 and 2) in *F. arundinacea* with P3/*Xba* I. Lane M: 1 kb λ ladder marker.

Table 11. Phenotypes, frequencies, and levels of phenotypic diversity detected in cpDNA for 53 *P. pratensis* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	Phenotypic diversity
P1/XB*	0.04	0.92	0.04		0.335
P2/XB	0.02	0.98			0.098
P3/XB	0.02	0.98			0.098
P4/BH	0.02	0.98			0.098
P4/RI	0.02	0.98			0.098
P4/XB	0.04	0.94	0.02		0.265
P6/RI	0.02	0.98			0.098
P7/RI	0.08	0.86	0.02	0.04	0.539
P7/XB	0.04	0.96			0.168
				Average	0.200

† Only polymorphic probe/enzyme combinations are listed.
* P1-P7 are probes from the barley chloroplast DNA library of Day and Ellis (1985); XB, BH, and RI represent the enzymes *Xba* I, *Bam* HI, and *Eco* RI, respectively.

with an average of 0.200. An example of three different phenotypes detected by P1/*Xba* I combination are shown in Fig. 2C (lanes 1 to 3). All three phenotypes had DNA fragments of 6.5, 3.1, 2.5, 0.7, 0.4, and 0.2 kb. Lane 2 (phenotype b) had an additional 14 kb fragment and was represented by 48 cultivars. Lane 1 (phenotype a) had two extra fragments of 14 and 8.1 kb. Lane 3 (phenotype c) had an additional 8.1 kb fragment. When combining all polymorphic probe/enzyme combinations, ten variable haplotypes (A through J, as shown in the last column of Table 12) were identified among the cpDNAs from the 53 *P. pratensis* cultivars.

Intraspecific cpDNA variation in genus Lolium

Fifty-two cultivars were examined in this species with the same set of clones and enzymes used for the other species. No intraspecific polymorphism was observed among the cultivars by Southern blot hybridization. An example of uniformity of DNA fragment banding pattern in this species with P4/*Xba* I is shown in Fig. 6.

Interspecific cpDNA variation

The high level of cpDNA RFLPs among most of the eight turfgrasses were revealed by all 10 cpDNA clones. Of the 40 probe/enzyme combinations, 38 were polymorphic, and only two failed to detect any polymorphism. The percentage of polymorphic probe/enzyme combinations found in each species and/or subspecies is shown in Fig. 7. Changes in the cpDNA fragment banding pattern are dependent upon the different probe/enzyme combinations. For example, a single combination of P1/*Xba* I revealed six different phenotypes, and could distinguish six turfgrasses. When combining more probe/enzyme combinations, all eight turfgrasses could be distinguished from each other. The average cpDNA phenotypic diversity observed among these eight turfgrasses is 1.222 (Table 13). The frequencies (F-values) of shared fragments among the eight distinct cpDNA genomes based upon the data from all 40 probe/enzyme combinations are summarized in Table 14. From this

Table 12. Haplotypes detected among cpDNAs of 53 *P. pratensis* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination									Chloroplast haplotype
	P1* XB	P2 XB	P3 XB	P4 BH	P4 RI	P4 XB	P6 RI	P7 RI	P7 XB	
Barzan	a¶	a	a	a	a	a	a	a	a	A
Gnome	a	a	a	a	a	a	a	b	a	B
P-104	a	a	a	a	a	a	a	b	a	B
Ram-1	a	a	a	a	a	a	a	b	a	B
Compact	a	a	a	a	b	b	a	b	a	C
Joy	a	a	a	a	a	a	a	b	a	B
Sydsport	a	a	a	a	a	a	a	b	a	B
Georgetown	a	a	a	a	a	a	a	b	a	B
Somerset	a	a	a	a	a	a	a	b	a	B
Able 1	a	a	a	a	a	a	a	b	a	B
Bar VB 577	a	a	a	a	a	a	a	b	a	B
Annika	b	a	a	a	a	a	a	a	a	D
Kenblue	a	a	a	a	a	a	a	b	a	B
Bristol	a	a	a	a	a	a	a	b	a	B
Ba 72-5000	a	a	a	a	a	a	a	a	a	A
Bar VB534	c	a	a	a	a	c	a	c	b	E
NE 80-88	b	a	a	a	a	a	a	a	a	D
America	a	a	a	a	a	a	a	b	a	B
Ba 69-82	a	a	a	a	a	a	a	b	a	B
Ba 73-54	a	a	a	a	a	a	a	b	a	B
Parade	a	a	a	a	a	a	a	b	a	B
HV 97	a	a	a	a	a	a	a	b	a	B
Cheri	a	a	a	a	a	a	a	b	a	B
Eclipse	a	a	a	a	a	a	a	b	a	B
Liberty	a	a	a	a	a	a	a	b	a	B
Destiny	a	a	a	a	a	a	a	b	a	B
Dawn	a	a	a	a	a	a	a	b	a	B
Merion	a	a	a	a	a	a	a	b	a	B
239(Suffolk)	a	a	a	a	a	a	a	b	a	B
Wabash	a	a	a	a	a	a	a	b	a	B

(Table 12 continued)

Cultivar	Probe/enzyme combination									Chloroplast haplotype
	P1 XB	P2 XB	P3 XB	P4 BH	P4 RI	P4 XB	P6 RI	P7 RI	P7 XB	
Ikone	a	a	a	a	a	a	a	b	a	B
F-1872	a	a	a	a	a	a	a	b	a	B
Aquila	c	a	a	a	a	c	a	c	a	F
K 1-152	a	a	a	a	a	a	a	b	a	B
Welcome	a	a	a	a	a	a	a	b	a	B
Rugby	a	a	a	a	a	a	a	b	a	B
Treton	a	a	a	a	a	a	a	b	a	B
Midnight	a	a	a	a	a	a	a	b	b	G
Challenger	a	a	a	a	a	a	a	b	a	B
Blacksburg	a	a	a	a	a	a	a	b	a	B
PST-CB 1	a	a	a	a	a	a	a	b	a	B
S.D. Certified	a	a	a	a	a	a	a	b	a	B
WW AG 468	a	a	a	a	a	a	a	b	a	B
Mom PP 2926	a	a	a	a	a	a	a	b	a	B
Bar VB 55	a	a	a	a	a	a	a	b	a	B
HV-96	a	a	a	a	a	a	a	b	a	B
Vantage	a	a	a	a	a	a	a	b	a	B
Classic	a	a	a	b	a	a	a	b	a	H
Ba 72-492	a	a	a	a	a	a	a	b	a	B
Ba 73-626	a	a	a	a	a	a	a	b	a	B
Amazon	a	b	b	a	a	a	a	b	a	I
Julia	a	a	a	a	a	a	a	b	a	B
WW AG 491	a	a	a	a	a	a	b	d	a	J

* P1-P7 are probes from the barley chloroplast DNA library of Day and Ellis (1985); XB, BH, and RI represent the enzymes *Xba* I, *Bam* HI, and *Eco* RI, respectively.
¶ a, b, and c represent the phenotypes detected by each corresponding probe/enzyme combination.

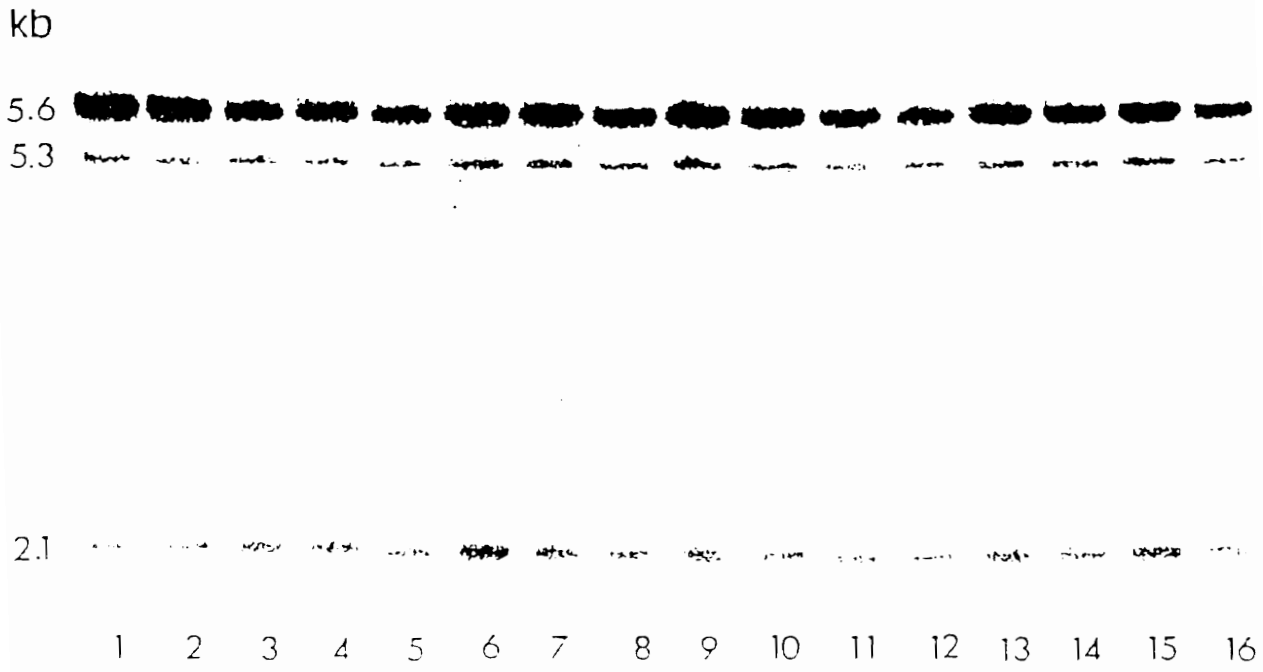

Fig. 6. Autoradiogram of Southern blot showing the uniformity (lanes 1-16) in *L. perenne* with P4/*Xba* I. Lane M: 1 kb λ ladder marker.

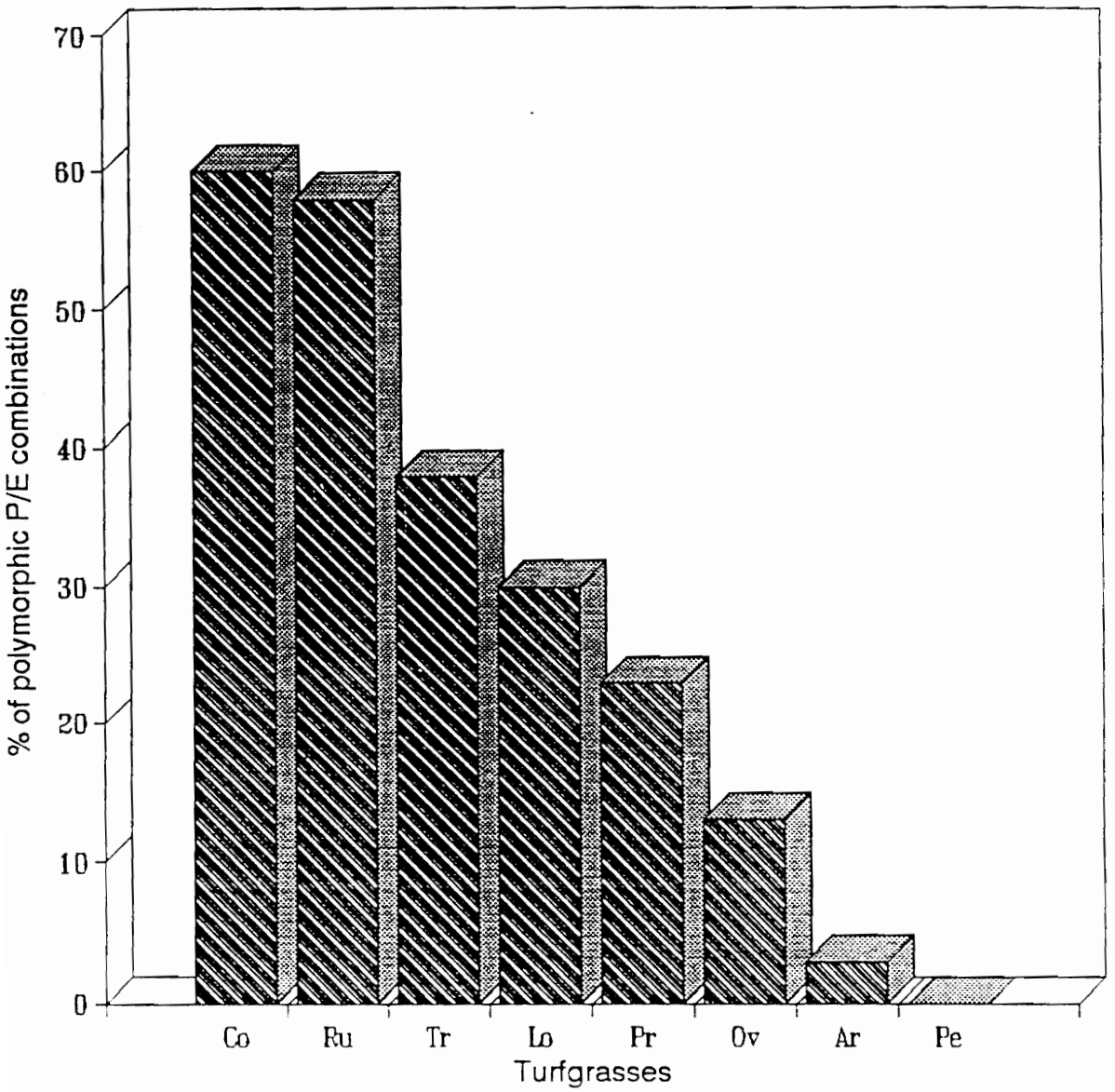

Fig. 7. Comparison of percentage of polymorphic probe/enzyme combinations detected in cpDNA. Co=*F. commutata*, Ru=*F. rubra*, Tr=*F. trichophylla*, Lo=*F. longifolia*, Pr=*P. pratensis*, Ov=*F. ovina*, Ar=*F. arundinacea*, and Pe=*L. perenne*.

Table 13. Phenotypes, frequencies, and levels of phenotypic diversity detected in cpDNA for the eight turfgrasses†.

Probe/enzyme combination	Phenotypes & their frequencies							Phenotypic diversity
	a	b	c	d	e	f	g	
P1/H3*	0.50	0.38	0.12					0.968
P1/BH	0.25	0.13	0.13	0.13	0257	0.11		1.730
P1/RI	0.25	0.25	0.13	0.25	0.12			1.557
P1/XB	0.13	0.13	0.13	0.13	0.13	0.25	0.10	1.901
P2/H3	0.63	0.25	0.12					0.891
P2/BH	0.50	0.13	0.13	0.13	0.11			1.384
P2/RI	0.13	0.25	0.25	0.25	0.12			1.557
P2/XB	0.75	0.13	0.12					0.735
P3/H3	0.38	0.13	0.13	0.25	0.11			1.487
P3/BH	0.38	0.25	0.13	0.13	0.11			1.487
P3/RI	0.38	0.25	0.25	0.12				1.314
P3/XB	0.38	0.25	0.13	0.13	0.11			1.487
P4/H3	0.63	0.25	0.12					0.891
P4/BH	0.38	0.13	0.13	0.13	0.13	0.10		1.658
P4/RI	0.25	0.25	0.13	0.13	0.13	0.11		1.730
P4/XB	0.25	0.25	0.13	025	0.12			1.557
P5/H3	0.63	0.25	0.12					0.891
P5/BH	0.38	0.13	0.13	0.25	0.11			1.487
P5/RI	0.75	0.13	0.12					0.735
P5/XB	0.38	0.25	0.25	0.12				1.314
P6/BH	0.63	0.25	0.12					0.891
P6/RI	0.38	0.25	0.25	0.12				1.314
P6/XB	0.38	0.25	0.25	0.12				1.314
P7/BH	0.50	0.13	0.25	0.12				1.211
P7/RI	0.38	0.25	0.13	0.13	0.11			1.487
P7/XB	0.38	0.38	0.24					1.079
P8/BH	0.63	0.25	0.12					0.891
P8/RI	0.12	0.25	0.25	0.38				1.314
P8/XB	0.13	0.25	0.25	0.25	0.12			1.557
P9/H3	0.63	0.37						0.659
P9/BH	0.63	0.13	0.13	0.11				1.064
P9/RI	0.38	0.25	0.25	0.12				1.314
P9/XB	0.25	0.25	0.13	0.25				1.557
P10/H3	0.75	0.13	0.12					0.735
P10/BH	0.88	0.12						0.366
P10/RI	0.63	0.25	0.12					0.891
P10/XB	0.38	0.13	0.13	0.25	0.12			1.487
							Average	1.222

† Only polymorphic probe/enzyme combinations are listed.
* P1-P10 are probes from the barley chloroplast DNA library of Day and Ellis (1985); H3, BH, RI, and XB represent the enzymes *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, respectively.

Table 14. Proportion (F-value) of shared fragments between each pair of the eight turfgrasses obtained by probing with ten cpDNA clones.

	Tr	Co	Lo	Ov	Ar	Pe	Pr
Ru*	0.76**	0.75	0.97	0.93	0.43	0.42	0.45
Tr		0.94	0.74	0.73	0.42	0.42	0.46
Co			0.73	0.65	0.41	0.41	0.47
Lo				0.95	0.36	0.42	0.45
Ov					0.41	0.41	0.44
Ar						0.89	0.50
Pe							0.48

* Ru=*F. rubra*, Tr=*F. trichophylla*, Co=*F. commutata*, Lo=*F. longifolia*, Ov=*F. ovina*, and Ar=*F. arundinacea*, Pe=*L. perenne*, and Pr=*P. pratensis*.
** Proportion (F-value) of shared fragments between each pair of the eight turfgrasses was calculated by the formula: F-value=2 Nxy/(Nx + Ny) (Nei and Li 1979).

table, it can be seen that the fraction of shared fragments among species ranged from 97% to 36%. The highest cpDNA similarity was between *F. rubra* and *F. longifolia* and between *F. longifolia* and *F. ovina*. A dendrogram drawn based on the data from Table 14 is shown in Fig. 8. The branching pattern (Fig. 8) shows two major groups in these eight turfgrasses based on the cpDNA analysis. The five turfgrasses (*F. rubra*, *F. trichophylla*, *F. commutata*, *F. longifolia*, and *F. ovina*) in the fine fescue subgenus, were clustered in the same group, while others were clustered in another group.

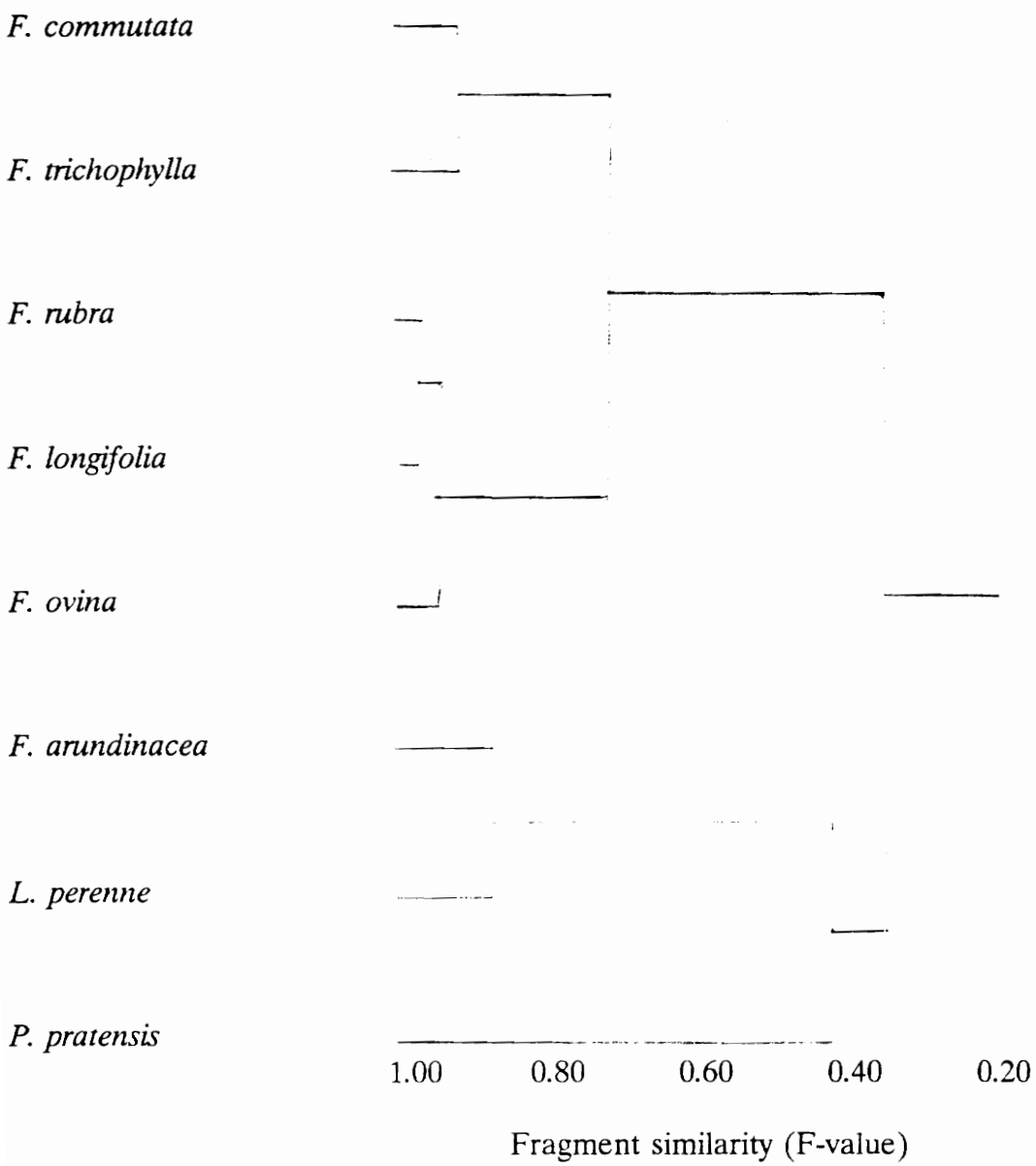

Fig. 8. A dendrogram showing the phylogenetic relationships among the eight turfgrasses in *Festuca*, *Lolium*, and *Poa* genera based on cpDNA RFLP analysis.

Discussion

The two sources (insertion/deletion and point mutation) of the observed RFLPs can be distinguished using one probe and several restriction enzymes. Length variation (insertion or deletion) is assumed if one can detect polymorphism with several enzymes and a single probe; whereas site variation (point mutation) is inferred if fragment length variation is observed with only one enzyme (e. g., Soltis *et al.* 1989a). Since specific changes were detected by several enzymes in our study, then most mutations are likely to be attributed to insertion/deletion events. Similar observations have been made for other species in different genera such as *Triticum* and *Aegilops* (Bowman et al. 1983), *Pisum* (Palmer et al. 1985a), *Glycine* (Apuya et al. 1988), *Oryza* (McCough et al. 1989), and *Plantago* (Hooglander et al. 1993). However, point mutations have been reported to be the most common type in other genera such as *Linum* (Coates and Cullis 1987), *Heuchera micrantha* (Soltis *et al.* 1989a), *Sorghum bicolor* (Duvall and Doebley 1990), and *Zea perennis* (Doebley 1989).

An average of 42, 30, 20, and 8% of the polymorphisms were detected by enzymes *Xba* I, *Eco* RI, *Bam* HI, and *Hind* III, respectively. The most polymorphic probes were P1, P3, and P4. Over 50% of the polymorphisms were revealed by these three clones. Probes P1, P4, and half of P3 are located in the large single-copy region of the barley chloroplast genome, and the remaining part of P3 is located in the inverted repeat region. This indicates that most mutations occur in the large single-copy and inverted repeat regions of turfgrass cpDNA. Almost all of the ten clones combined with each of the enzymes *Xba* I and *Eco* RI and these provided the highest number of different RFLP patterns. Thus, these two enzymes detected a high level of variation and were best suited for RFLP analysis of cpDNA variation in the turfgrasses examined.

Comparisons of cpDNA variation among turfgrasses

Extensive levels of intraspecific cpDNA diversity were observed in all six turfgrasses of *Festuca* except *F. arundinacea*. The highest degree of intraspecific cpDNA variation was detected in *F. rubra* (phenotypic diversity value of 0.868), and the highest frequency of polymorphic probe/enzyme combinations was found in *F. commutata* (60%) and *F. rubra* (58%). In addition, the maximum number of cpDNA phenotypes detected was four, as was observed in each of *F. rubra*, *F. commutata*, and *F. longifolia*. In contrast, the lowest cpDNA variability was observed within *F. arundinacea* (phenotypic diversity value of 0.227), which had only one-fourth of the variability of *F. rubra*. Furthermore, *F. arundinacea* had the lowest percentage of polymorphic probe/enzyme combinations (only 2.5%). These data exemplify that a wide range of variation exists among genera in *Festuca*.

A relatively low level of intraspecific cpDNA polymorphism was found among the 53 cultivars in *P. pratensis*. Only ten haplotypes were distinguishable by combining all polymorphic probe/enzyme combinations.

No differences were found among the 52 cultivars of *L. perenne* examined. This uniformity in intraspecific cpDNA is indicative of a greater degree of cytoplasmic homogeneity within this species as compared to other species in this study. However, previous studies with isozymes have detected variation among cultivars of this species (Ferguson and Grabe 1986; Nielsen *et al.* 1985; Hayward and McAdam 1977; and Gilliland *et al.* 1982). More recently, a high level of nuclear DNA polymorphism was found among five cultivars of *L. perenne* (Xu et al. 1992). The uniformity of cpDNA indicates that there may be little cytoplasmic diversity among cultivars in this species. The size of the chloroplast genome and its highly conserved gene sequences may explain this lack of diversity (Palmer *et al.* 1985a). In addition, minor differences in the chloroplast genome may not be detectable by Southern hybridization using cpDNA as probes. DNA sequencing, a more sensitive technique, may be more useful for the identification of such minor variations.

Species relationships based on cpDNA data

F-values were used as indices of relatedness to elucidate the relationships of these turfgrasses. Similarity of cpDNA was observed to be highest between *F. rubra* and *F. longifolia* and between *F. longifolia* and *F. ovina*. The higher proportion (F-value) of shared fragments of cpDNA among these species is likely to be indicative of a greater degree of cytoplasmic genetic homogeneity, and suggests that they are closely related and may have a common origin.

As shown in Fig. 8, the five turfgrasses in the fine fescue subgenus were clustered in the same group, while others were clustered in a different group. These observations are in agreement with the traditional classification (Turgeon 1985). However, when considering the two subgroups within the fine fescue subgenus (Fig. 8), it is found that our observations are in slight disagreement with the traditional classification. For example, from Fig. 8 and Table 14, it can be seen that *F. rubra* is more closely related to *F. longifolia* and *F. ovina* than to *F. commutata* and *F. trichophylla*. Similarity to the latter two species is expected according to the traditional classification. The minor disagreements observed between the results from cpDNA and the traditional classification may be explained by 1) the limited number of samples studied in some species, 2) different types of markers (e. g., cpDNA vs morphological), and 3) detection of nonchloroplast DNA sequences. Since *F. trichophylla* and *F. commutata* are in the same subgroup, this suggests they are closely related to each other and may have a common origin. The classification of the fine fescue subgenus based on cpDNA data is also slightly different from that based on the traditional methods.

Based on morphological and cytological studies, *F. arundinacea* and *L. perenne* have traditionally been classified in two different genera (Turgeon 1985). However, analysis based on cpDNA indicates that *F. arundinacea* is more closely related to *L. perenne* than the other species or genera of this study (Fig. 8). For example, an F-value of 0.89 was obtained between *F. arundinacea* and *L. perenne*, while F-values

ranged from 0.36 to 0.43 between *F. arundinacea* and other species or genera. These results are in agreement with the observation of Webster and Buckner (1970) that *F. arundinacea* would hybridize with *L. perenne* but not with other species. The close relationship of the cytoplasm for these two genera suggests that they may have a common origin. However, these genera exhibit extreme differences in both morphology (e.g. plant type and leaf shape) and basic chromosome number (*F. arundinacea*, 2n=6x=42; *L. perenne*, 2n=2x=14).

F-values between *P. pratensis* and the seven other turfgrasses ranged from 0.44 to 0.50, indicating a rather distant relationship. These results are in agreement with morphological dissimilarities between *P. pratensis* and the other turfgrasses (Turgeon 1985). Apparently, this species has a different cytoplasmic origin than the others, which may be related to its restrictive reproductive mechanism (apomixis). In contrast, Ohmura et al. (1993) reported that *P. pratensis* was closely related to *Festuca* but their conclusion was based on a limited study of restriction endonuclease analysis of cpDNA in two accessions of each genera. Other independent studies on mtDNA and/or nuclear DNA are therefore required.

Summary

This study represents the first large-scale cpDNA RFLP analysis in cultivated turfgrasses designed to determine the level of variation both within and between species. The results indicate that there is extensive intraspecific cpDNA variation in most members of the *Festuca* genus, but that a relatively low level of cpDNA polymorphism exists within *P. pratensis*, and that cpDNA is uniform within *L. perenne*. Phylogenetic relationships among the eight turfgrasses from the analysis of cpDNA indicate that *F. arundinacea* and *L. perenne* are closely related to each other. *P. pratensis* has a rather low degree of relationship to any of the turfgrasses in the *Festuca* and *Lolium* genera. This research further demonstrates that cpDNA is useful as a molecular marker for species and subspecies identification. Estimation of

genetic diversity and germplasm identification based on cpDNA analysis can provide useful information in facilitating the conservation of germplasm and the development of breeding programs. Such applications include the use of cpDNA diversity as a criterion in germplasm collection as well as in elimination of duplicates in gene bank samples. cpDNA markers can also be used in the generation of populations with a higher level of cytoplasmic diversity and in determining the mode of inheritance (paternal/maternal) of organellar DNA genomes.

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Chapter II

Introduction

Mitochondria are important in plant respiration and metabolic activity. In addition, some very important traits, such as cytoplasmic male sterility, are dependent upon the expression of mitochondrial genes. RFLPs of mitochondrial DNA (mtDNA) have been widely used as molecular genetic markers to investigate genetic variation, phylogenetic relationships, and evolution at the intraspecific level in animals (Avise *et al.* 1987; DeSalle and Giddings 1986; Hale and Singh 1986; Harrison 1989; Moritz *et al.* 1987; Kambhampati and Rai 1991). In these studies, extensive mtDNA variation within and between populations has been detected and utilized as genetic markers in providing important insight into population dynamics, speciation, geographic variation, introgressive hybridization, and phylogenetic relationships in animal species.

In contrast, research on plant mtDNA has lagged behind that of both animal mtDNA and plant cpDNA. In plants, cpDNA has been broadly used as a genetic marker to study cytoplasmic genetic diversity, biogeography, phylogenetic and evolutionary processes (Palmer, 1985a,b; Birky 1988; Clegg 1989; Crawford 1990; see also Chapter I). Relatively few studies on intraspecific variation in plant mtDNA have been reported. The level of nucleotide substitution in mtDNA is much lower in plants than in animals and even up to four times lower than in cpDNA (Wolfe *et al.* 1987; Palmer and Herbon 1988). Some specific features which have hindered studies of plant mtDNA variation are that the plant mitochondrial genome is large and variable in size and complexity. The plant mitochondrial genomes also undergo rapid structural changes during evolution. Highly variable restriction fragment patterns make it difficult to directly interpret restriction enzyme patterns from total mitochondrial genomic DNA (Sederoff 1987). The variation in restriction patterns detected by the Southern hybridization method with mtDNA probes has been used to overcome these limitations (Bland *et al.* 1985; Chowdhury and Smith 1988; Graur

et al. 1989; Breiman 1987; Breiman *et al.* 1991; Grabau *et al.* 1992; Terachi and Tsunewaki 1992). Several studies on species phylogenetic relationships and cytoplasmic male sterility have been conducted using RFLP analysis of mtDNA (McLean and Hanson 1986; Chowdhury and Smith 1988; Smith *et al.* 1989; Khairallah *et al.* 1991; Terachi and Tsunewaki 1992, Rouwendal *et al.* 1992). Palmer and Herbon (1988) observed that the level of variability in mtDNA was about one-fourth of that in cpDNA among *Brassica* species. In contrast, McLean and Hanson (1986) found that mtDNA in *Lycopersicon* species appeared to be evolving more rapidly than cpDNA, and a similar conclusion was made for *Hordeum vulgare* and *H. spontaneum* by Holwerda *et al.* (1986). The variability detected in cultivated rice species was two times higher in mtDNA than in cpDNA (Ishii *et al.* 1993). So far, comparative data on mtDNA variation within and between closely related species of higher plants is limited, and it remains unclear whether mtDNA will be as useful as cpDNA for phylogenetic and evolutionary studies. To evaluate the value of mtDNA as a marker for addressing these questions, it is necessary to conduct extensive studies in different plant species (Clegg 1989). Turfgrasses have received much less attention than other crops in studies on genetic diversity at the DNA level. No study of intra- and inter-specific mtDNA variability has been reported. In this study, RFLP analysis has been used to evaluate and determine the amount of intra- and inter-specific mtDNA variation by Southern hybridization using the same set of plant materials as in the cpDNA analysis. The levels of mtDNA and cpDNA diversity for these turfgrasses are compared and their relationships are inferred by combining the data from both cpDNA and mtDNA.

Materials and Methods

Plant materials

Plant materials used in this survey were the same as in the study on cpDNA variation described in Chapter I (Chapter I, Table 1).

Source of mtDNA clones

No mitochondrial genomic DNA library of turfgrasses is available. However, in conducting a preliminary study with four cultivars randomly chosen from each species and subspecies, it was found that a set of 19 wheat mtDNA clones (probes) generously provided by Dr. P. Covello (Dalhousie University, Canada) exhibited a high degree of homology with turfgrass DNA and detected significant variation. Plasmids containing these 19 inserts were amplified either by transforming into DH5 *alpha* competent cells as described in Chapter I or by the polymerase chain reaction (PCR) technique following the manufacturer's (Perkin-Elmer) instructions. Amplification reactions were in a total volume of 50 μl and contained 10 X PCR buffer, 25 mM $MgCl_2$, 1.25 mM dATP (deoxy adenosine triphosphate), dCTP (deoxy cytidine triphosphate), dGTP (deoxy guanosine triphosphate), and dTTP (deoxy thymidine triphosphate), 20 mM of each primer, 25 ng plasmid DNA, and 1 unit of *Taq* DNA polymerase covered with one drop of mineral oil. Amplification was performed in a DNA Thermal Cycler programmed for 35 cycles of three minutes at 94 ^{0}C, one minute at 37 ^{0}C, and two minutes at 72 ^{0}C. Plasmid DNA amplified by the method of transformation was extracted as described in Chapter I.

DNA preparation, RFLP assays, and data analysis

The approaches used for DNA isolation, restriction enzyme digestion, electrophoresis, Southern blot hybridization, autoradiography, and data analysis were the same as for the study on cpDNA variation as described in Chapter I.

Results

Intraspecific mtDNA variation in genus Festuca

mtDNA variation in *F. rubra*--RFLPs were revealed by all 19 mtDNA clones used in this survey with at least one of the four restriction enzymes tested (Table 1). Of the 76 probe/enzyme combinations surveyed, 60 were polymorphic. Nine of the 19 mtDNA clones detected polymorphism with all four enzymes, six with three enzymes, two with two enzymes, and two with one enzyme. Extensive mtDNA variation was detected, and phenotypic diversity values ranged from 0.487 to 1.420 with an average of 0.891. Up to five phenotypes were detected by Southern hybridization in each of five probe/enzyme combinations (Table 1). As in the cpDNA study, the mtDNA fragment banding patterns in the autoradiography indicate that most of the mutations most likely can be attributed to insertion/deletion events. Fig. 1 shows an example of an insertion/deletion polymorphic fragment pattern revealed by the 7/*Bam* HI combination (mtDNA clone 7 and *Bam* HI restriction enzyme). Five DNA fragment banding patterns (phenotypes) were detected. Lanes 1 through 5 all had DNA fragments of 5.5 and 3.9 kb. Lane 2 (phenotype b) had additional fragments of 15 and 11.5 kb and represents 56% of the total cultivars in this subspecies. In contrast, lane 1 (phenotype a) had two fragments of 11 and 14 kb in addition to the 15 and 11.5 kb fragments; lane 3 (phenotype c) had three fragments of 14, 11, and 8 kb and was missing the 15 and 11.5 kb bands, lane 4 (phenotype d) had fragments of 14 and 11 kb but no 15 and 11.5 kb fragments, while lane 5 (phenotype e) only had a fragment of 11 kb. As shown in the last column of Table 2, the mitochondrial genomes of 16 cultivars in *F. rubra* based on seven polymorphic probe/enzyme combinations are classified into 15 haplotypes, A through O. Only two cultivars, Franklin and Sylvester, had the same fragment pattern and could not be distinguished from one another.

mtDNA variation in *F. commutata*--RFLPs were detected with all 19 mtDNA

Table 1. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for 16 *F. rubra* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	Phenotypic diversity
Prol*/BH**	0.19	0.56	0.25			0.987
Prol/XB	0.06	0.69	0.25			0.771
pQT12/H3	0.07	0.64	0.29			0.831
PQT12/BH	0.06	0.63	0.25	0.06		0.975
pQT12/RI	0.06	0.69	0.25			0.771
pQT12/XB	0.07	0.67	0.07	0.06	0.13	1.074
H465/H3	0.07	0.73	0.20			0.738
H465/BH	0.08	0.53	0.31	0.08		1.103
H465/RI	0.07	0.60	0.13	0.20		1.079
H465/XB	0.06	0.56	0.25	0.13		1.105
7/H3	0.44	0.50	0.06			0.876
7/BH	0.13	0.56	0.06	0.19	0.06	1.244
7/RI	0.13	0.63	0.13	0.11		1.064
7/XB	0.08	0.67	0.25			0.816
pFC5-2/BH	0.06	0.69	0.25			0.771
pFC5-2/RI	0.25	0.75				0.562
pFC5-2/XB	0.13	0.62	0.25			0.907
476/BH	0.19	0.50	0.25	0.06		1.177
476/RI	0.37	0.63				0.659
476/XB	0.13	0.62	0.25			0.907
pYT2-4/XB	0.07	0.64	0.29			0.831
Tyr/XB	0.13	0.62	0.25			0.907
pHJ271/BH	0.13	0.62	0.25			0.907
pHJ271/RI	0.25	0.75				0.562
pHJ271/XB	0.06	0.69	0.25			0.771
490/BH	0.06	0.69	0.13	0.12		0.944
490/RI	0.06	0.69	0.25			0.771
490/XB	0.07	0.71	0.22			0.762
B30/H3	0.38	0.62				0.664
B30/BH	0.19	0.56	0.25			0.987
B30/RI	0.13	0.62	0.19	0.06		1.046
B30/XB	0.13	0.67	0.20			0.855
H458/BH	0.13	0.62	0.25			0.907

(Table 1 continued)

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	Phenotypic diversity
H458/RI	0.33	0.67				0.634
H458/XB	0.13	0.62	0.25			0.907
B342/H3	0.13	0.62	0.25			0.907
B342/BH	0.13	0.62	0.25			0.907
B342/RI	0.13	0.62	0.06	0.19		1.046
B342/XB	0.13	0.62	0.25			0.907
H454/H3	0.31	0.44	0.25			1.070
H454/BH	0.21	0.79				0.514
H454/RI	0.13	0.56	0.25	0.06		1.105
H454/XB	0.19	0.50	0.31			1.025
Sal5.6b/BH	0.56	0.06	0.19	0.13	0.06	1.244
Sal5.6b/RI	0.07	0.60	0.07	0.26		1.028
Sal5.6b/XB	0.15	0.38	0.08	0.31	0.08	1.420
B376/H3	0.07	0.67	0.13	0.13		0.984
B376/BH	0.06	0.63	0.25	0.06		0.975
B376/RI	0.31	0.69				0.619
B376/XB	0.06	0.69	0.25			0.771
H469/RI	0.13	0.62	0.25			0.907
H469/XB	0.43	0.57				0.683
Pst24/H3	0.38	0.62				0.664
Pst24/BH	0.69	0.31				0.619
Pst24/RI	0.13	0.56	0.13	0.12.	0.06	1.278
Pst24/XB	0.07	0.60	0.07	0.26		1.028
114/H3	0.19	0.81				0.487
114/BH	0.13	0.68	0.19			0.843
114/RI	0.06	0.69	0.25			0.771
114/XB	0.07	0.67	0.27			0.807

Average 0.891

† Only polymorphic probe/enzyme combinations are listed.

* Probes are from the wheat mtDNA library.

** BH, H3, RI, and XB represent the enzymes *Bam* HI, *Hind* III, *Eco* RI, and *Xba* I, respectively.

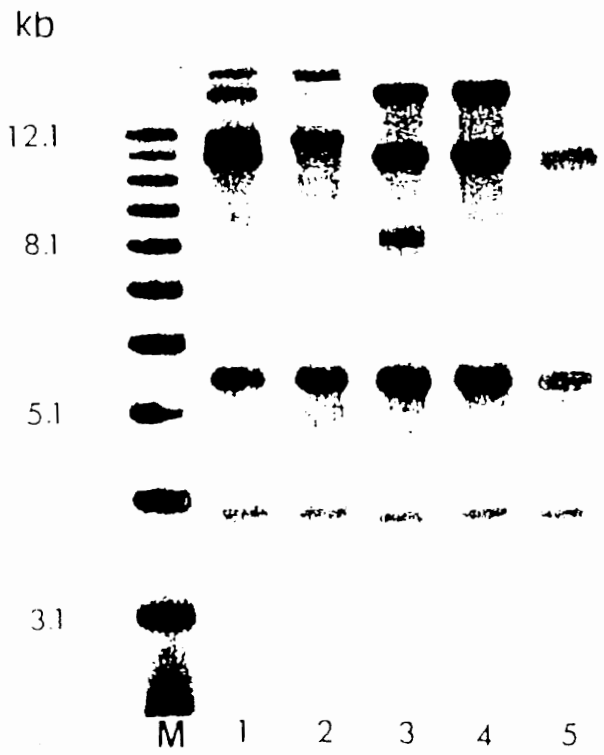

Fig. 1. Autoradiogram of Southern blot showing five mtDNA phenotypes in *F. rubra* with 7/*Bam* HI (lanes 1-5). Lane M: 1 kb λ ladder marker.

Table 2. Seven polymorphic probe/enzyme combinations detected the maximum number of haplotypes among mtDNA of 16 *F. rubra* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination							Mitochondrial haplotype
	7* H3**	7 BH	PH RI	H4 RI	B2 BH	PST RI	PST XB	
Elanor	a¶	a	a	a	a	a	a	A
Franklin	b	b	a	b	b	b	b	B
Sylvester	b	b	a	b	b	b	b	B
WW RS 130	b	b	a	b	b	b	c	C
ZW 42-158	a	c	b	c	c	c	d	D
PST-4NI	b	b	a	b	b	b	b	E
Bargena	a	b	a	d	b	b	b	F
Bar FR8RC3	a	b	a	b	b	b	b	G
Cindy	a	d	b	c	c	d	d	H
Boreal	b	b	a	b	b	b	b	I
WW RS 138	a	b	a	b	b	b	b	J
PST-4C8	c	e	a	b	b	b	b	K
Herald	a	d	b	c	c	d	d	L
Longfellow	a	d	b	c	c	c	d	M
Ensylva	b	a	a	a	d	a	b	N
Jasper	b	b	a	b	b	e	a	O

* 7, PH, H4, B2, and PST represent the wheat mtDNA probes 7, pHJ2-7-1, H454, B376, and Pst24, respectively.

** H3, BH, RI, and XB represent the enzymes *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, respectively.

¶ a, b, c, and d represent the phenotypes detected by each corresponding probe/enzyme combination.

probes with at least one of the four enzymes used. As shown in Table 3, nine of the 19 polymorphic probes demonstrated polymorphism with all four enzymes, five with three enzymes, two with two enzymes, and three were polymorphic for only one enzyme. The highest diversity value was 1.229 and the lowest was 0.227 with an average of 0.488. Altogether, 74% of the total probe/enzyme combinations were polymorphic in this subspecies. Most polymorphic probe/enzyme combinations revealed two to three phenotypes, and some of them identified up to five different phenotypes. The maximum number of haplotypes were revealed when the data from five polymorphic probe/enzyme combinations were combined. This combined analysis identified 13 haplotypes (A through M) among the mtDNAs of the 17 cultivars in this subspecies (Table 4).

mtDNA variation in *F. trichophylla*--Polymorphisms were detected with all 19 mtDNA probes with at least one of the four enzymes tested. As shown in Table 5, five of the 19 mtDNA clones detected polymorphism with all four enzymes, six with three enzymes, five with two enzymes, and three with one enzyme. A total of 51 polymorphic probe/enzyme combinations were observed. Most of the polymorphic combinations could detect two to three phenotypes. Phenotypic frequencies and diversity estimates are shown in Table 5. The diversity values ranged from 0.347 to 1.529 with an average of 0.759. As shown in the last column of Table 6 (A through I), the mitochondrial genomes of all nine cultivars in the subspecies of *F. trichophylla* are distinguishable from each other using the four polymorphic probe/enzyme combinations.

mtDNA variation in *F. longifolia*--Intraspecific DNA variability was identified in the mitochondrial genome of *F. longifolia*. All 19 probes detected variation within this species with at least one of the four enzymes assayed. Extensive polymorphisms were detected, and the range of phenotypic diversity values were from 0.456 to 1.555 with an average of 0.923 (Table 7). Of 56 polymorphic probe/enzyme combinations, 19 detected two to three phenotypes, and 9 combinations each revealed four to five

Table 3. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for 17 *F. commutata* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	Phenotypic diversity
Prol*/BH**	0.88	0.12				0.366
Prol/XB	0.94	0.06				0.227
pQT12/H3	0.86	0.07	0.07			0.502
pQT12/BH	0.75	0.06	0.13	0.06		0.829
pQT12/RI	0.93	0.07				0.253
pQT12/XB	0.81	0.07	0.06	0.06		0.695
H465/H3	0.82	0.12	0.06			0.586
H465/BH	0.87	0.07	0.06			0.476
H465/RI	0.94	0.06				0.227
H465/XB	0.82	0.06	0.06	0.06		0.670
7/H3	0.94	0.06				0.227
7/BH	0.94	0.06				0.227
7/RI	0.82	0.12	0.06			0.585
7/XB	0.80	0.13	0.07			0.630
pFC5-2/BH	0.36	0.43	0.14	0.07		1.192
476/BH	0.81	0.13	0.06			0.605
476/RI	0.94	0.06				0.227
476/XB	0.94	0.06				0.227
pYT2-4/XB	0.77	0.15	0.08			0.688
Tyr/XB	0.80	0.13	0.07			0.630
pHJ271/BH	0.94	0.06				0.227
490/BH	0.81	0.13	0.06			0.605
490/RI	0.82	0.06	0.06	0.06		0.670
490/XB	0.80	0.07	0.07	0.06		0.740
B30/H3	0.93	0.07				0.253
B30/BH	0.94	0.06				0.227
B30/RI	0.25	0.38	0.25	0.06	0.06	1.229
B30/XB	0.93	0.07				0.253
H458/BH	0.88	0.12				0.366
H458/RI	0.94	0.06				0.227
H458/XB	0.94	0.06				0.227
B342/H3	0.88	0.06	0.06			0.450
B342/BH	0.88	0.12				0.366

(Table 3 continued)

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	Phenotypic diversity
B342/RI	0.88	0.06	0.06			0.450
B342/XB	0.94	0.06				0.227
H454/H3	0.76	0.06	0.12	0.06		0.801
H454/BH	0.53	0.40	0.07			0.889
H454/RI	0.53	0.06	0.23	0.12	0.06	1.266
H454/XB	0.47	0.47	0.06			0.879
Sal5.6b/BH	0.82	0.12	0.06			0.586
Sal5.6b/RI	0.88	0.06	0.06			0.450
Sal5.6b/XB	0.93	0.07				0.253
B376/H3	0.70	0.18	0.06	0.06		0.897
B376/BH	0.88	0.06	0.06			0.450
B376/RI	0.88	0.06	0.06			0.450
B376/XB	0.81	0.13	0.06			0.605
H469/RI	0.88	0.06	0.06			0.450
H469/XB	0.93	0.07				0.253
Pst24/H3	0.94	0.06				0.227
Pst24/BH	0.94	0.06				0.227
Pst24/RI	0.93	0.07				0.253
Pst24/XB	0.81	0.07	0.06	0.06		0.695
114/H3	0.94	0.06				0.227
114/BH	0.94	0.06				0.227
114/RI	0.88	0.06	0.06			0.450
114/XB	0.94	0.06				0.227
					Average	0.488

† Only polymorphic probe/enzyme combinations are listed.
* Probes are from the wheat mtDNA library.
** BH, H3, RI, and XB represent the enzymes *Bam* HI, *Hind* III, *Eco* RI, and *Xba* I, respectively.

Table 4. Five polymorphic probe/enzyme combinations detected the maximum number of haplotypes among mtDNA of 17 *F. commutata* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination					Mitochondrial haplotype
	H4* *Hind* III	H4 *Eco* RI	B3 *Hind* III	B1 *Eco* RI	H5 *Eco* RI	
NK 82492	a¶	a	a	a	a	A
Molinda	a	a	a	a	a	A
Barnica	a	a	a	a	a	A
Shadow	b	a	a	b	a	B
Atlanta	a	a	b	c	a	C
Mary	a	a	b	c	a	C
Jamestown	a	a	a	b	a	D
Waldorf	c	b	a	d	a	E
Epsom	c	c	c	d	a	F
N-105	a	c	a	b	a	G
Raymond	a	d	a	a	a	H
Estoril	a	c	a	c	a	I
Wilma	a	d	a	a	b	J
BAR FR 9F	a	c	d	c	a	K
Capitol	d	e	b	b	a	L
Enjoy	a	a	a	b	a	M
Kolet	a	a	a	b	a	M

* H4, B3, B1, and H5 represent the wheat mtDNA probes H454, B376, B30, and H458, respectively.

¶ a, b, c, d, and e represent the phenotypes detected by each corresponding probe/enzyme combination.

Table 5. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for nine *F. trichophylla* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	Phenotypic diversity
Prol*/BH**	0.89	0.11				0.347
Prol/XB	0.78	0.22				0.527
pQT12/H3	0.75	0.13	0.12			0.735
pQT12/BH	0.63	0.25	0.12			0.891
pQT12/RI	0.78	0.11	0.11			0.680
pQT12/XB	0.38	0.50	0.12			0.968
H465/H3	0.75	0.13	0.12			0.735
H465/BH	0.67	0.22	0.11			0.844
H465/RI	0.89	0.11				0.347
H465/XB	0.38	0.38	0.12	0.12		1.244
7/BH	0.78	0.11	0.11			0.680
7/RI	0.23	0.33	0.33	0.11		1.313
7/XB	0.13	0.13	0.63	0.11		1.064
pFC5-2/BH	0.13	0.50	0.25	0.12		1.211
pFC5-2/XB	0.89	0.11				0.347
476/BH	0.63	0.25	0.12			0.891
476/XB	0.89	0.11				0.347
pYT2-4/XB	0.88	0.12				0.366
Tyr/XB	0.88	0.12				0.366
pHJ271/BH	0.29	0.71				0.602
pHJ271/RI	0.29	0.71				0.602
pHJ271/XB	0.29	0.71				0.602
490/BH	0.50	0.50				0.692
490/RI	0.45	0.33	0.11	0.11		1.211
490/XB	0.13	0.13	0.63	0.11		1.064
B30/H3	0.89	0.11				0.347
B30/BH	0.88	0.12				0.366
B30/RI	0.88	0.12				0.366
B30/XB	0.88	0.12				0.366
H458/XB	0.89	0.11				0.347
B342/H3	0.44	0.44	0.12			0.976
B342/BH	0.11	0.78	0.11			0.680
B342/RI	0.43	0.14	0.29	0.14		1.272

(Table 5 continued)

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	Phenotypic diversity
B342/XB	0.89	0.11				0.347
H454/H3	0.89	0.11				0.347
H454/RI	0.25	0.50	0.13	0.12		1.211
H454/XB	0.75	0.25				0.562
Sal5.6b/BH	0.11	0.78	0.11			0.680
Sal5.6b/RI	0.43	0.14	0.29	0.14		1.272
Sal5.6b/XB	0.63	0.13	0.12	0.12		1.064
B376/H3	0.11	0.22	0.22	0.12	0.33	1.529
B376/BH	0.22	0.33	0.12	0.33		1.319
B376/RI	0.13	0.13	0.74			0.753
B376/XB	0.25	0.50	0.25			1.038
H469/RI	0.78	0.22				0.527
H469/XB	0.67	0.22	0.11			0.844
Pst24/BH	0.78	0.11	0.11			0.680
Pst24/RI	0.75	0.25				0.562
Pst24/XB	0.14	0.14	0.44	0.14	0.14	1.461
114/RI	0.75	0.13	0.12			0.735
114/XB	0.88	0.12				0.366
					Average	0.759

† Only polymorphic probe/enzyme combinations are listed.
* Probes are from the wheat mtDNA library.
** BH, H3, RI, and XB represent the enzymes *Bam* HI, *Hind* III, *Eco* RI, and *Xba* I, respectively.

Table 6. Four polymorphic probe/enzyme combinations detected the maximum number of haplotypes among mtDNA of nine *F. trichophylla* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination				Mitochondrial haplotype
	B2* *Hind* III	B2 *Xba* I	B3 *Hind* III	B3 *Bam* HI	
Bar FR 9P	a¶	a	a	a	A
Barlotte	a	a	b	b	B
Barcrown	a	a	c	c	C
FRT-30149	b	a	b	b	D
HF 102	b	a	e	d	E
Marker	a	a	c	a	F
LD 3488	b	a	c	b	G
ZW 42-160	b	a	e	d	H
HF 138	c	b	e	d	I

* B2, and B3 represent the wheat mtDNA probes B342 and B376, respectively.
¶ a, b, c, d, and e represent the phenotypes detected by each corresponding probe/enzyme combination.

Table 7. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for six *F. longifolia* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	Phenotypic diversity
Prol*/XB**	0.33	0.50	0.17			1.013
pQT12/H3	0.33	0.33	0.17	0.17		1.334
pQT12/BH	0.13	0.75	0.12			0.735
pQT12/RI	0.17	0.50	0.17	0.16		1.241
pQT12/XB	0.17	0.34	0.17	0.16	0.16	1.555
H465/H3	0.17	0.17	0.34	0.16	0.16	1.555
H465/BH	0.17	0.17	0.34	0.16	0.16	1.555
H465/RI	0.17	0.17	0.34	0.16	0.16	1.555
H465/XB	0.17	0.50	0.17	0.16		1.241
7/BH	0.20	0.40	0.20	0.20		1.333
7/RI	0.17	0.17	0.50	0.16		1.241
7/XB	0.20	0.20	0.20	0.20	0.20	1.610
pFC5-2/BH	0.83	0.17				0.456
pFC5-2/RI	0.50	0.34	0.16			1.006
476/BH	0.67	0.17	0.16			0.862
476/RI	0.83	0.17				0.456
476/XB	0.67	0.17	0.16			0.862
pYT2-4/RI	0.17	0.67	0.16			0.862
pYT2-4/XB	0.83	0.17				0.456
Tyr/RI	0.17	0.67	0.16			0.862
Tyr/XB	0.83	0.17				0.456
pHJ271/BH	0.50	0.34	0.16			1.006
pHJ271/RI	0.83	0.17				0.456
pHJ271/XB	0.50	0.34	0.16			1.006
490/BH	0.83	0.17				0.456
490/RI	0.17	0.50	0.17	0.16		1.241
490/XB	0.34	0.17	0.17	0.16	0.16	1.555
B30/H3	0.67	0.17	0.16			0.862
B30/BH	0.83	0.17				0.456
B30/XB	0.17	0.50	0.17	0.16		1.241
H458/RI	0.67	0.17	0.16			0.862
H458/XB	0.83	0.17				0.456
B342/H3	0.83	0.17				0.456

(Table 7 continued)

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	Phenotypic diversity
B342/BH	0.83	0.17				0.456
B342/RI	0.67	0.17	0.16			0.862
B342/XB	0.83	0.17				0.456
H454/H3	0.83	0.17				0.456
H454/BH	0.83	0.17				0.456
H454/RI	0.83	0.17				0.456
H454/XB	0.67	0.33				0.634
Sal5.6b/BH	0.83	0.17				0.456
Sal5.6b/RI	0.83	0.17				0.456
Sal5.6b/XB	0.40	0.20	0.20	0.20		1.333
B376/H3	0.34	0.17	0.17	0.16	0.16	1.555
B376/BH	0.34	0.50	0.16			1.006
B376/RI	0.33	0.33	0.17	0.17		1.334
B376/XB	0.34	0.17	0.17	0.16	0.16	1.555
H469/RI	0.67	0.17	0.16			0.862
H469/XB	0.60	0.20	0.20			0.950
Pst24/H3	0.83	0.17				0.456
Pst24/BH	0.67	0.17	0.16			0.862
Pst24/RI	0.17	0.17	0.34	0.16	0.16	1.555
Pst24/XB	0.20	0.60	0.20			0.950
114/BH	0.83	0.17				0.456
114/RI	0.67	0.17	0.16			0.862
114/XB	0.50	0.34	0.16			1.006
					Average	0.923

† Only polymorphic combinations are listed.
* Probes are from the wheat mtDNA library.
** BH, H3, RI, and XB represent the enzymes *Bam* HI, *Hind* III, *Eco* RI, and *Xba* I, respectively.

phenotypes. All six cultivars in this species are distinguishable from each other based on two polymorphic probe/enzyme combinations (Table 8).

mtDNA variation in *F. ovina*--Only two cultivars were examined in *F. ovina*. RFLPs were observed by 12 of the 76 probe/enzyme combinations. These two cultivars are distinguishable by Southern hybridization with any one of the probe/enzyme combinations (Table 9).

mtDNA variation in *F. arundinacea*--RFLPs of mtDNA were revealed by only three clones, and a total of six probe/enzyme combinations could detect polymorphism. Phenotypic frequencies and diversity values are summarized in Table 10. Using *Bam* HI digests, clone 7 revealed three phenotypes among the 53 cultivars in this species (Fig. 2). Lanes 1 through 3 all had a DNA fragment of 5.5 kb. Lane 2 (phenotype b) had additional fragments of 8.3 and 7.5 kb and represents 35 of the total cultivars studied in this species. Lane 3 (phenotype c), represented by 14 cultivars, had only the 8.3 kb fragment. Lane 1 (phenotype a), represented by four cultivars, had a 7.5 kb fragment and was missing the 8.3 kb fragment. When combining the data from all six polymorphic probe/enzyme combinations, 16 haplotypes (A through P as shown in the last column of Table 11) were identified among the 53 cultivars in the species of *F. arundinacea*.

Intraspecific mtDNA variation in genus Lolium

RFLPs of mtDNA among the 50 cultivars in *L. perenne* were revealed by four of the 19 clones studied. Although the number of polymorphic probe/enzyme combinations was relatively low (11 of the total 76 combinations), a high degree of diversity was observed, and the range of phenotypic diversity values was from 0.292 to 1.424 with an average of 0.903 (Table 12). Eight phenotypes were detected by one clone with Southern hybridization. Fig. 3 (lanes 1 to 8) shows an example of polymorphism detected by the B376/*Xba* I combination. Lanes 1 through 8 all had several common *Xba* I fragments of 18, 11, 8.2, 7.5, 3.8, and 3.3 kb. Lane 5

Table 8. Two polymorphic probe/enzyme combinations detected the maximum number of haplotypes among mtDNA of six *F. longifolia* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination		Mitochondrial haplotype
	Pst24* *Bam* HI	Pst24 *Eco* RI	
Serra	a¶	a	A
Malody	a	b	B
Attila	b	c	C
Aurora	a	c	D
Biljart	c	d	E
Valda	a	e	F

* Pst24 is the wheat mtDNA probe.
¶ a, b, c, d, and e represent the phenotypes detected by each corresponding probe/enzyme combination.

Table 9. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for two *F. ovina* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	Phenotypic diversity
H465*/H3**	0.50	0.50	0.692
H465/BH	0.50	0.50	0.692
7/BH	0.50	0.50	0.692
7/RI	0.50	0.50	0.692
7/XB	0.50	0.50	0.692
pHJ271/BH	0.50	0.50	0.692
490/RI	0.50	0.50	0.692
490/XB	0.50	0.50	0.692
Sal5.6b/XB	0.50	0.50	0.692
B376/BH	0.50	0.50	0.692
H469/RI	0.50	0.50	0.692
Pst24/XB	0.50	0.50	0.692
		Average	0.692

† Only polymorphic probe/enzyme combinations are listed.
* Probes are from the wheat mtDNA library.
** H3, BH, RI, and XB represent the enzymes *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, respectively.

Table 10. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for 53 *F. arundinacea* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	Phenotypic diversity
7*/H3**	0.48	0.43	0.09	0.932
7/BH	0.06	0.67	0.27	0.790
7/RI	0.67	0.33		0.634
Pst24/H3	0.31	0.59	0.10	0.904
Pst24/BH	0.36	0.26	0.38	1.086
114/BH	0.36	0.13	0.51	0.976
			Average	0.887

† Only polymorphic probe/enzyme combinations are listed.
* Probes are from the wheat mtDNA library.
** BH, H3, RI, and XB represent the enzymes *Bam* HI, *Hind* III, *Eco* RI, and *Xba* I, respectively.

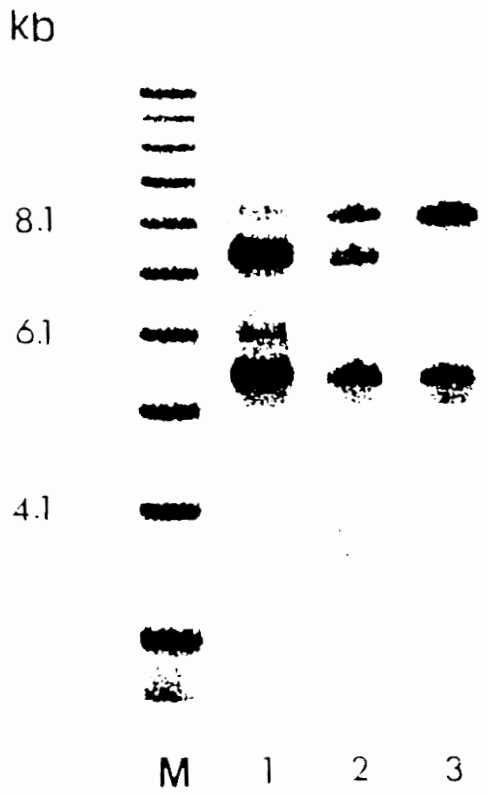

Fig. 2. Autoradiogram of Southern blot showing two mtDNA phenotypes (lanes 1-3) in *F. arundinacea* with 7/*Bam* HI. Lane M: 1 kb λ ladder marker.

Table 11. Haplotypes revealed among mtDNA of 53 *F. arundinacea* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination						Mitochondrial haplotype
	7* H3**	7 BH	7 RI	Pst H3	Pst BH	114 BH	
Adventure	a¶	a	a	a	a	a	A
Trident	b	b	b	b	b	b	B
Titan	c	c	b	c	b	b	C
Pick DDF	b	b	b	b	c	c	D
Pick 127	c	c	b	c	b	b	C
Pick SLD	b	b	b	b	c	c	D
PE-7E	c	c	b	c	b	b	C
Hubbard 87	a	a	a	a	a	a	A
Legend	a	a	a	a	a	a	A
Taurus	b	b	b	b	c	c	D
Sundance	b	b	b	b	c	c	D
Fatima	a	b	b	b	a	c	E
Normarc 25	b	b	b	b	c	c	D
Willamette	a	b	b	b	a	c	E
Chieftain	a	a	a	a	a	a	A
Thoroughbred	a	b	a	a	a	a	F
Pick TF9	b	b	b	a	c	c	G
PST-50L	a	b	b	b	c	c	H
PST-5D7	b	b	b	b	c	c	D
Cimmaron	b	b	b	b	c	c	D
Bonanza	b	b	b	b	c	c	D
PST-5AG	a	a	a	a	a	c	I
PST-5BL	a	a	a	a	a	a	A
PST-5MW	b	b	b	b	c	c	D
Trailblaze	a	b	b	b	a	a	J
Jaguar	a	a	a	a	a	a	A
PST-DBC	c	c	b	c	b	b	C
Olympic	a	a	a	a	a	a	A
Jaguar II	a	a	a	a	a	a	A
Apache	b	b	b	b	c	c	D

(Table 11 continued)

Cultivar	Probe/enzyme combination						Mitochondrial haplotype
	7 H3	7 BH	7 RI	PST24 H3	PST24 BH	114 BH	
PST-5DM	a	b	a	a	a	a	F
Pick DM	a	a	a	a	a	a	A
Pacer	b	b	b	b	c	c	D
Carefree	b	b	b	b	c	c	D
Richmond	b	b	b	b	c	c	D
Tip	a	b	a	b	c	a	K
KY-31	b	b	b	c	c	b	L
Bel 86-1	b	b	b	b	c	c	D
PST-5EN	b	b	b	b	a	a	M
F-lawn5GL	c	c	b	c	b	b	C
Rebel	a	a	a	a	a	a	A
Tribute	b	b	b	b	b	c	N
Arid	b	b	b	b	c	c	D
Wrangler	a	a	a	a	a	a	A
Mesa	b	b	b	b	b	c	N
JB-2	a	a	a	a	a	a	A
Falcon	b	b	b	b	c	c	D
Syn GA	b	b	b	b	b	c	N
Pick GH6	b	b	b	b	b	c	N
PST-5HF	b	b	b	b	b	c	N
Monarch	a	b	a	b	b	a	O
Normarc 99	c	b	b	b	b	c	P
PST-5F2	b	b	b	b	b	c	N

* 7, Pst24, and 114 are the wheat mtDNA probes.

** H3, BH, and RI represent the enzymes *Hind* III, *Bam* HI, and *Eco* RI, respectively.

¶ a, b, c, d, and e represent the phenotypes detected by each corresponding probe/enzyme combination.

Table 12. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for 50 *L. perenne* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies								Phenotypic diversity
	a	b	c	d	e	f	g	h	
pQT12*/BH**	0.02	0.18	0.69	0.09	0.02				0.938
pQT12/RI	0.92	0.04	0.02	0.02					0.362
pQT12/XB	0.94	0.02	0.02	0.02					0.292
B342/H3	0.27	0.10	0.61	0.02					0.963
B342/BH	0.37	0.55	0.08						0.899
B342/XB	0.12	0.10	0.10	0.68					0.976
H454/XB	0.16	0.72	0.08	0.02	0.02				0.888
B376/H3	0.30	0.06	0.60	0.02	0.02				0.992
B376/BH	0.29	0.55	0.02	0.04	0.02	0.08			1.175
B376/RI	0.08	0.28	0.56	0.08					1.085
B376/XB	0.30	0.04	0.50	0.02	0.06	0.04	0.02	0.02	1.368
								Average	0.903

† Only polymorphic probe/enzyme combinations are listed.
* Probes are from the wheat mtDNA library.
** BH, H3, RI, and XB represent the enzymes *Bam* HI, *Hind* III, *Eco* RI, and *Xba* I, respectively.

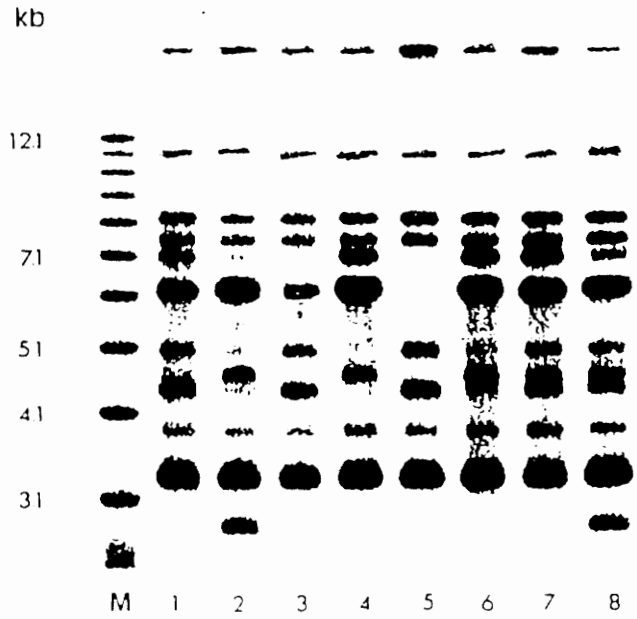

Fig. 3. Autoradiogram of Southern blot showing eight mtDNA phenotypes (lanes 1-8) in *L. perenne* with B376/*Xba* I. Lane M: 1 kb λ ladder marker.

contained additional fragments of 5.1 and 4.5 kb, and this fragment banding pattern represents 25 of the 52 cultivars in this species. Unlike lane 5, lane 1 (15 cultivars) had extra fragments of 7 and 6.1 kb; lane 3 (three cultivars) had an additional fragment of 6.1 kb; lane 2 (one cultivar) had three fragments of 6.1, 4.8, and 2.8 kb and was missing the 5.1 and 4.5 kb bands; lane 4 (two cultivars) had three fragments of 7, 6.1 and 4.8 kb and lacked the 5.1 and 4.5 kb fragments; lane 6 (two cultivars) had three fragments of 7, 6.1 and 4.8 kb but did not have the 4.5 kb fragment, while lane 7 (one cultivar) had three additional fragments of 7, 6.1, and 4.8. Lane 8 (one cultivar) had four extra fragments of 7, 6.1, 4.8, 2.8 kb, respectively. From Fig. 3, it also can be seen that most of the insertion/deletion events occurred in the region between 4.5 to 7 kb. When combining the data from all polymorphic probe/enzyme combinations, a total of 29 haplotypes (A through AC as shown in the last column of Table 13) were identified among the mtDNAs for the 50 cultivars of *L. perenne*.

Intraspecific mtDNA variation in genus Poa

Fifty-three cultivars in the species of *P. pratensis* were examined. RFLPs were revealed by nine of the 19 mtDNA clones used in this survey. Twenty-two polymorphic probe/enzyme combinations were found among these cultivars with at least one of the four restriction enzymes tested. Phenotypes, frequencies, and estimated phenotypic diversity values are summarized in Table 14. Diversity values ranged from 0.098 to 1.198 with an average of 0.461, and as many as seven different phenotypes were detected by the *Xba* I digests when probing with the clone pHJ271. When combining data from all polymorphic probe/enzyme combinations, the mitochondrial genomes of 53 cultivars in *P. pratensis* can be classified into 29 haplotypes (A through AC), as shown in the last column of Table 15. Two major haplotypes, namely haplotypes C and K are represented by 16 and six cultivars, respectively.

Table 13. Haplotypes detected among mtDNA of 50 *L. perenne* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination										Mitochondrial haplotype
	PQ* BH**	PQ RI	PQ XB	B2 H3	B2 BH	B2 XB	H1 XB	B3 H3	B3 BH	B3 RI	B3 XB	
Barry	a¶	a	a	a	a	a	a	a	a	a	a	A
Barlp 410	b	a	a	b	a	b	a	a	a	a	b	B
Yorktowni	c	a	a	a	a	c	b	a	a	b	a	C
Palmer	c	a	a	c	b	d	b	c	b	c	c	D
Diplomat	b	b	a	a	a	d	b	a	a	b	a	E
Pavo	d	a	a	b	c	b	c	b	c	d	d	F
Caliente	c	a	a	c	b	d	b	c	b	c	e	G
Aquarius	c	a	a	c	b	d	b	c	b	c	c	D
Goalie	c	a	a	c	b	d	b	c	b	c	e	G
Acrobat	d	a	a	b	c	b	c	d	d	d	b	H
Brenda	c	a	a	c	b	d	b	c	b	c	c	D
Derby	c	a	a	c	b	d	b	c	b	c	c	D
Gator	d	b	a	b	c	b	c	b	e	d	b	I
Patriot	c	a	a	c	b	d	b	c	b	c	c	D
Rodeo	c	a	a	c	b	d	b	c	b	c	c	D
Allaire	b	a	a	d	a	c	a	a	a	a	f	J
Pick 300	c	a	a	c	b	d	b	c	b	c	c	D
Ovation	d	a	a	b	c	b	c	b	d	d	b	K
SR 4000	c	a	a	c	b	d	b	c	b	c	c	D
SR 4031	b	a	a	a	a	d	b	a	a	b	a	L
Pick 647	c	a	a	c	b	d	b	c	b	c	c	D
Ranger	b	a	a	a	a	c	a	c	a	b	a	M
ISI-K2	c	a	a	a	a	d	b	a	f	b	a	D
Pennfine	c	a	a	c	b	d	b	c	b	c	e	G
PSU-222	c	a	a	a	a	c	b	a	f	b	a	N
Sheriff	c	c	a	c	b	d	b	c	b	c	c	O
Birdie II	c	a	b	c	b	d	b	c	b	c	c	P
Regency	c	a	a	c	a	d	b	c	f	b	a	Q
PST-2PM	c	a	a	c	b	d	b	c	b	c	c	D
PST-2DD	e	a	a	c	b	d	b	c	b	c	c	S

(Table 13 continued)

Cultivar	Probe/enzyme combination											Mitochondrial haplotype
	PQ BH	PQ RI	PQ XB	B2 H3	B2 BH	B2 XB	H4 XB	B3 H3	B3 BH	B3 RI	B3 XB	
PST-250	c	a	c	c	b	d	b	c	b	c	c	T
Vintage-2DF	c	a	a	c	b	d	b	c	b	c	c	D
PST-259	c	a	a	c	b	d	b	c	b	c	c	D
PST-M2E	c	a	a	c	b	d	b	c	b	c	c	D
246	c	a	a	c	b	d	b	c	b	c	c	D
PST-2HH	c	a	a	c	b	d	b	c	b	c	c	D
ISI-851	b	a	a	a	a	a	b	a	a	b	a	U
Manhattan	c	a	a	a	a	a	d	a	a	b	a	V
Repell	c	d	d	c	b	d	b	c	b	c	c	W
Del 946	b	a	a	a	a	a	b	a	a	b	a	U
J 207	b	a	a	a	a	a	a	d	f	a	g	X
J 208	c	a	a	c	b	d	e	c	b	c	c	Y
Linn	c	a	a	c	b	d	b	c	b	c	c	D
Runaway	c	a	a	a	a	d	a	a	a	b	a	Z
Cowboy	c	a	a	c	b	d	b	c	b	c	c	D
Delray	c	a	a	a	a	a	a	a	a	b	a	AA
Pick 600	c	a	a	c	b	d	b	c	b	c	c	D
ManhattanII	c	a	a	a	a	d	b	a	a	b	h	AB
Omega II	c	a	a	c	b	d	b	c	b	c	c	D
Delle	c	a	a	a	a	c	a	a	a	b	a	AC

* PQ, B2, H1, and B3 represent the mtDNA clones pQT12, B342, H454, and B376, respectively.

** BH, RI, XB, and H3 represent the enzymes *Bam* HI, *Eco* RI, *Xba* I, and *Hind* III, respectively.

¶ a, b, c, d, e, f, g, and h represent the phenotypes detected by each corresponding probe/enzyme combination.

Table 14. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for 53 *P. pratensis* cultivars†.

Probe/enzyme combination	Phenotypes & their frequencies							Phenotypic diversity
	a	b	c	d	e	f	g	
Prol*/BH**	0.98	0.02						0.098
Prol/XB	0.86	0.06	0.04	0.02	0.02			0.584
pQT12/BH	0.66	0.04	0.02	0.08	0.16	0.04		1.105
pQT12/RI	0.96	0.02	0.02					0.195
pQT12/XB	0.90	0.02	0.02	0.06				0.420
H465/XB	0.98	0.02						0.098
7/BH	0.94	0.06						0.227
7/XB	0.92	0.02	0.04	0.02				0.362
pHJ271/H3	0.69	0.19	0.06	0.06				0.910
pHJ271/BH	0.94	0.06						0.227
pHJ271/RI	0.81	0.19						0.487
pHJ271/XB	0.17	0.02	0.63	0.07	0.07	0.02	0.02	1.198
B30/RI	0.94	0.02	0.02	0.02				0.292
B30/XB	0.79	0.15	0.02	0.02	0.02			0.705
H454/RI	0.90	0.04	0.04	0.02				0.431
Sal5.6b/BH	0.96	0.06						0.227
Sal5.6b/RI	0.96	0.06						0.227
Sal5.6b/XB	0.90	0.06	0.02	0.02				0.420
B376/H3	0.92	0.06	0.02					0.324
B376/BH	0.92	0.06	0.02					0.324
B376/RI	0.25	0.65	0.02	0.06	0.02			0.951
B376/XB	0.92	0.06	0.02					0.324
							Average	0.461

† Only polymorphic probe/enzyme combinations are listed.
* Probes are from the wheat mtDNA library.
** BH, H3, RI, and XB represent the enzymes *Bam* HI, *Hind* III, *Eco* RI, and *Xba* I, respectively.

Table 15. Haplotypes detected among mtDNA of 53 *P. pratensis* cultivars by their Southern hybridization.

Cultivar	Probe/enzyme combination																					Mitochondrial haplotype
	P1* BH**	P1 XB	PQ BH	PQ RI	PQ XB	H1 XB	7 BH	7 XB	PH H3	PH BH	PH RI	PH XB	B1 EI	B1 XB	H4 RI	Sa BH	Sa RI	Sa XB	B3 H3	B3 BH	B3 RI	B3 XB	
Berzan	a¶	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	A
Gnome	b	a	b	a	a	b	a	a	a	a	a	b	b	a	a	a	a	a	a	a	b	a	B
P-104	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Ram-1	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Compact	a	b	c	b	a	a	a	b	b	b	b	a	a	b	a	a	a	a	a	a	c	a	D
Joy	a	a	d	a	a	a	b	c	c	a	b	d	a	b	b	a	a	a	a	a	a	a	E
Sydsport	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Georgetown	a	a	a	a	a	a	a	a	d	a	a	a	a	a	a	a	a	a	a	a	b	a	F
Somerset	a	a	a	a	a	a	a	a	d	a	a	a	a	b	a	a	a	a	a	a	b	a	G
Able 1	a	a	a	a	a	a	a	a	d	a	a	a	a	a	a	a	a	a	a	a	a	a	H
Bar VB 577	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Annika	c	c	d	a	a	a	a	d	a	a	a	c	a	b	a	a	a	a	b	b	d	b	I
Kenblue	a	a	d	a	a	a	b	c	c	a	b	d	a	b	b	a	a	b	a	a	a	a	J
Bristol	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	a	a	K
Ba 72 5000	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Bar VB 534	a	d	a	a	a	a	a	a	d	a	b	a	a	b	c	a	a	a	b	b	d	b	I
Ne80 88	a	c	b	a	b	a	a	a	d	a	a	a	a	a	a	b	b	c	a	a	a	a	M
America	a	a	f	a	a	a	a	a	d	a	b	a	c	a	a	a	a	a	a	a	a	a	N
Ba 69-82	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	O
Ba 73 540	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Parade	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	P
HV 97	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Cheri	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Eclipse	a	a	a	a	c	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	Q
Liberty	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Destiny	a	a	a	a	a	a	a	a	d	a	a	c	a	a	a	a	a	a	a	a	a	a	R
Dawn	a	a	a	a	d	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	S
Merion	a	a	a	a	d	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	a	T
239(Suffolk)	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Wabash	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	U
Ikone	a	a	a	c	a	a	a	a	c	a	a	f	a	b	a	a	a	a	a	a	a	a	V
F-1872	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Aquila	a	a	a	a	a	a	a	a	d	a	b	a	a	b	c	b	b	c	b	b	d	b	W
K 1-152	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	a	X
Welcome	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	U
Rugby	a	a	a	a	d	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	S
Trenton	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	U
Midnight	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	U
Challenger	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Blacksburg	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
PST-CB 1	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
S D Certified	a	a	a	a	a	a	a	a	d	a	b	d	a	a	a	a	a	a	a	a	a	a	Y
WW AG 455	a	a	f	a	a	a	a	a	a	a	a	d	a	a	a	a	a	a	a	a	a	a	Z
M PP 2926	a	a	a	a	a	a	a	a	b	a	b	a	a	c	a	a	a	a	a	a	a	a	AA
Bar VB 55	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
HV 96	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	b	a	C
Vantage	a	a	a	a	a	a	a	a	d	a	b	g	a	d	a	a	a	a	a	a	a	a	AB
Classic	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	a	a	K
Ba 72 492	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	a	a	K
Ba73 626	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	a	a	K
Amazon	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	a	a	K
Julia	a	a	a	a	a	a	a	a	a	a	a	c	a	a	a	a	a	a	a	a	a	a	K
WW AG 491	a	a	a	a	a	a	a	a	b	b	b	a	d	a	d	a	b	d	c	c	a	c	AC

* P1, PQ, H1, 7, PH, B1, H4, Sa, B3, and Ps represent the wheat mtDNA clones Prol, pQT12, H465, 7, pHJ271, B30, Sal5.6b, B376, and Pst24, respectively.
** BH, XB, RI, and H3 represent the enzymes *Bam* HI, *Xba* I, *Eco* RI, and *Hind* III, respectively.
¶ a, b, c, d, e, f, and g represent the phenotypes detected by each corresponding probe/enzyme combination.

Interspecific mtDNA variation

Extensive cytoplasmic polymorphisms among eight turfgrasses were revealed by all 19 mtDNA clones. As shown in Table 16, 72 of the 76 probe/enzyme combinations were polymorphic, and nine detected up to eight phenotypes which distinguished all the eight turfgrasses examined. For example, the single combination of H465/*Eco* RI revealed eight distinct phenotypes among the 208 samples. Each phenotype corresponded to only one of the eight species and subspecies, thus every one of the eight turfgrasses could be identified (Table 16). The range of phenotypic diversity values are from 0.386 to 2.076 with an average of 1.489. A high percentage of probe/enzyme combinations are polymorphic for *F. rubra, F. commutata, F. trichophylla*, and *F. longifolia*, as shown in Fig. 4. The frequencies (F-values) of shared fragments among the eight distinct mtDNA genomes based upon the data from all 76 probe/enzyme combinations are summarized in Table 17. From this table, it can be seen that the fraction of shared fragments ranged from 25% to 91%. The two subspecies with the highest F-value (0.91) were *F. trichophylla* and *F. commutata*. A dendrogram drawn based on the data from Table 17 is shown in Fig. 5. The branching pattern (Fig. 5) shows the three major groups in these eight turfgrasses based on the mtDNA analysis.

Polymorphisms detected by mtDNA and cpDNA

A higher percentage of polymorphic probe/enzyme combinations and levels of intraspecific variation were detected in mtDNA, as compared to cpDNA, in all eight turfgrasses except *F. ovina* (Fig. 4 and 6). The phenotypic diversity value detected in *F. arundinacea* was three times higher for mtDNA than for cpDNA (Fig. 6). Similar observations were made in comparisons of polymorphisms detected by both mtDNA and cpDNA in *Oryza sativa* and *O. glaberrima* (Ishii *et al.* 1993), *Lycopersicom* (McLean and Hanson 1986), and *Hordeum vulgare* and *H. spontaneum* (Holwerda *et al.* 1986). In addition, a higher percentage of haplotypes were detected

Table 16. Phenotypes, frequencies, and levels of phenotypic diversity detected in mtDNA for eight turfgrasses in *Festuca*, *Lolium*, and *Poa* genera†.

Probe/enzyme combination	Phenotypes & their frequencies								Phenotypic diversity
	a	b	c	d	e	f	g	h	
Prol*/H3**	0.75	0.25							0.562
Prol/BH	0.38	0.25	0.13	0.12	0.12				1.487
Prol/XB	0.38	0.13	0.13	0.12	0.12	0.12			1.660
pQT12/H3	0.29	0.29	0.14	0.14	0.14				1.543
pQT12/BH	0.13	0.25	0.25	0.13	0.12	0.12			1.730
pQT12/RI	0.13	0.25	0.13	0.13	0.12	0.12	0.12		1.903
pQT12/XB	0.25	0.13	0.13	0.13	0.12	0.12	0.12		1.903
H465/H3	0.13	0.25	0.13	0.13	0.12	0.12	0.12		1.903
H465/BH	0.20	0.40	0.40						1.056
H465/RI	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	2.076
H465/XB	0.13	0.25	0.25	0.13	0.12	0.12			1.730
7/H3	0.38	0.25	0.13	0.12	0.12				1.487
7/BH	0.13	0.25	0.13	0.13	0.12	0.12	0.12		1.903
7/RI	0.25	0.13	0.13	0.13	0.12	0.12	0.12		1.903
7/XB	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	2.076
pFC5-2/H3	0.62	0.13	0.13	0.12					1.080
pFC5-5/BH	0.13	0.13	0.13	0.25	0.12	0.12	0.12		1.903
pFC5-2/RI	0.50	0.13	0.13	0.12	0.12				1.384
pFC5-2/XB	0.38	0.25	0.13	0.12	0.12				1.487
476/H3	0.62	0.13	0.12	0.12					1.080
476/BH	0.25	0.13	0.13	0.13	0.12	0.12	0.12		1.903
476/RI	0.25	0.25	0.13	0.13	0.12	0.12			1.730
476/XB	0.13	0.25	0.13	0.13	0.12	0.12	0.12		1.903
pYT2-4/BH	0.62	0.25	0.13						0.907
pYT2-4/RI	0.38	0.13	0.13	0.12	0.12	0.12			1.660
pYT2-4/XB	0.25	0.13	0.13	0.13	0.12	0.12	0.12		1.903
Tyr/BH	0.87	0.13							0.386
Tyr/RI	0.38	0.13	0.13	0.12	0.12	0.12			1.660
Tyr/XB	0.25	0.25	0.13	0.13	0.12	0.12			1.730
pHJ2-7-1/H3	0.75	0.13	0.12						0.735
pHJ2-7-1/BH	0.25	0.25	0.13	0.13	0.12	0.12			1.730
pHJ2-7-1/RI	0.38	0.25	0.13	0.12	0.12				1.487
pHJ2-7-1/XB	0.38	0.25	0.13	0.12	0.12				1.487
490/H3	0.25	0.38	0.13	0.12	0.12				1.487
490/BH	0.25	0.13	0.13	0.13	0.12	0.12	0.12		1.903
490/RI	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	2.076
490/XB	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	2.076
B30/H3	0.38	0.25	0.13	0.12	0.12				1.487
B30/BH	0.38	0.25	0.13	0.12	0.12				1.487

(Table 16 continued)

Probe/enzyme combination	Phenotypes & their frequencies a	b	c	d	e	f	g	h	Phenotypic diversity
B30/RI	0.38	0.13	0.13	0.12	0.12	0.12			1.660
B30/XB	0.25	0.25	0.13	0.13	0.12	0.12			1.730
H458/H3	0.62	0.13	0.13	0.12					1.080
H458/BH	0.38	0.25	0.13	0.12	0.12				1.487
H458/RI	0.38	0.25	0.13	0.12	0.12				1.487
H458/XB	0.38	0.38	0.12	0.12					1.244
B342/H3	0.38	0.13	0.13	0.12	0.12	0.12			1.660
B342/BH	0.38	0.25	0.13	0.12	0.12				1.487
B342/RI	0.25	0.13	0.13	0.13	0.12	0.12	0.12		1.903
B342/XB	0.38	0.25	0.13	0.12	0.12				1.487
H454/H3	0.25	0.25	0.13	0.13	0.12	0.12			1.730
H454/BH	0.25	0.13	0.13	0.13	0.12	0.12	0.12		1.903
H454/RI	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.1	2.076
H454/XB	0.38	0.13	0.13	0.12	0.12	0.12			1.660
Sal5.6b/H3	0.62	0.13	0.13	0.12					1.080
Sal5.6b/BH	0.38	0.25	0.13	0.12	0.12				1.487
Sal5.6b/RI	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.1	2.076
Sal5.6b/XB	0.13	0.25	0.13	0.13	0.12	0.12	0.12		1.903
B376/H3	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	2.076
B376/BH	0.38	0.25	0.13	0.12	0.12				1.487
B376/RI	0.25	0.25	0.13	0.13	0.12	0.12			1.730
B376/XB	0.13	0.25	0.13	0.13	0.12	0.12	0.12		1.903
H469/BH	0.62	0.13	0.13	0.12					0.080
H469/RI	0.13	0.25	0.13	0.13	0.12	0.12	0.12		0.903
H469/XB	0.38	0.13	0.13	0.12	0.12	0.12			0.660
Pst24/H3	0.38	0.25	0.13	0.12	0.12				1.487
Pst24/BH	0.13	0.13	0.13	0.25	0.12	0.12	0.12		1.903
Pst24/RI	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	2.076
Pst24/XB	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	2.076
114/H3	0.25	0.62	0.13						0.907
114/BH	0.25	0.25	0.13	0.13	0.12	0.12			1.730
114/RI	0.13	0.25	0.25	0.13	0.12	0.12			1.730
114/XB	0.13	0.25	0.13	0.12	0.25	0.12			1.730
								Average	1.489

† Only polymorphic probe/enzyme combinations are listed.
* Probes are from the wheat mtDNA library.
** H3, BH, RI, and XB represent the enzymes *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, respectively.

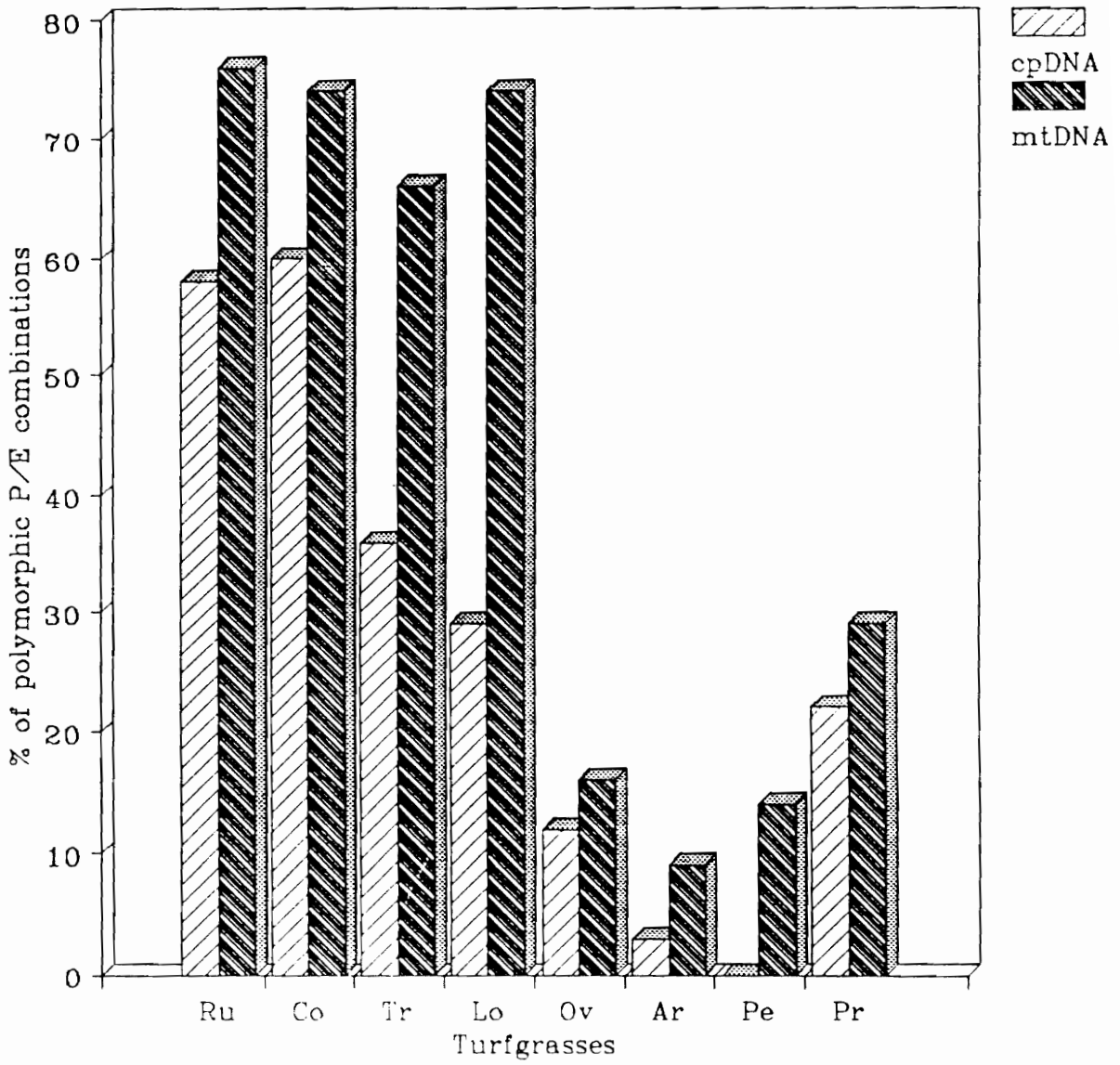

Fig. 4. Comparison of the percentage of polymorphic probe/enzyme combinations detected in cpDNA and mtDNA. Ru=*F. rubra*, Co=F. *commutata*, Tr=*F. trichophylla*, Lo=*F. longifolia*, Ov=*F. ovina*, Ar=*F. arundinacea*, Pe=*L. perenne*, and Pr=*P. pratensis*.

Table 17. Proportion (F-value) of shared fragments between each pair of the eight turfgrasses obtained by probing with 19 mtDNA clones.

	Tr*	Co	Lo	Ov	Ar	Pe	Pr
Ru	0.52**	0.50	0.86	0.81	0.26	0.25	0.29
Tr		0.91	0.52	0.48	0.30	0.31	0.28
Co			0.52	0.48	0.30	0.30	0.30
Lo				0.82	0.28	0.28	0.28
Ov					0.28	0.27	0.30
Ar						0.43	0.29
Pe							0.26

* Ru=*F. rubra*, Tr=*F. trichophylla*, Co=*F. commutata*, Lo=*F. longifolia*, Ov=*F. ovina*, Ar=*F. arundinacea*, Pe=*L. perenne*, and Pr=*P. pratensis*.

** Proportion (F-value) of shared fragments between two turfgrasses, F-value=2 $N_{xy}/(N_x + N_y)$ (Nei and Li 1979).

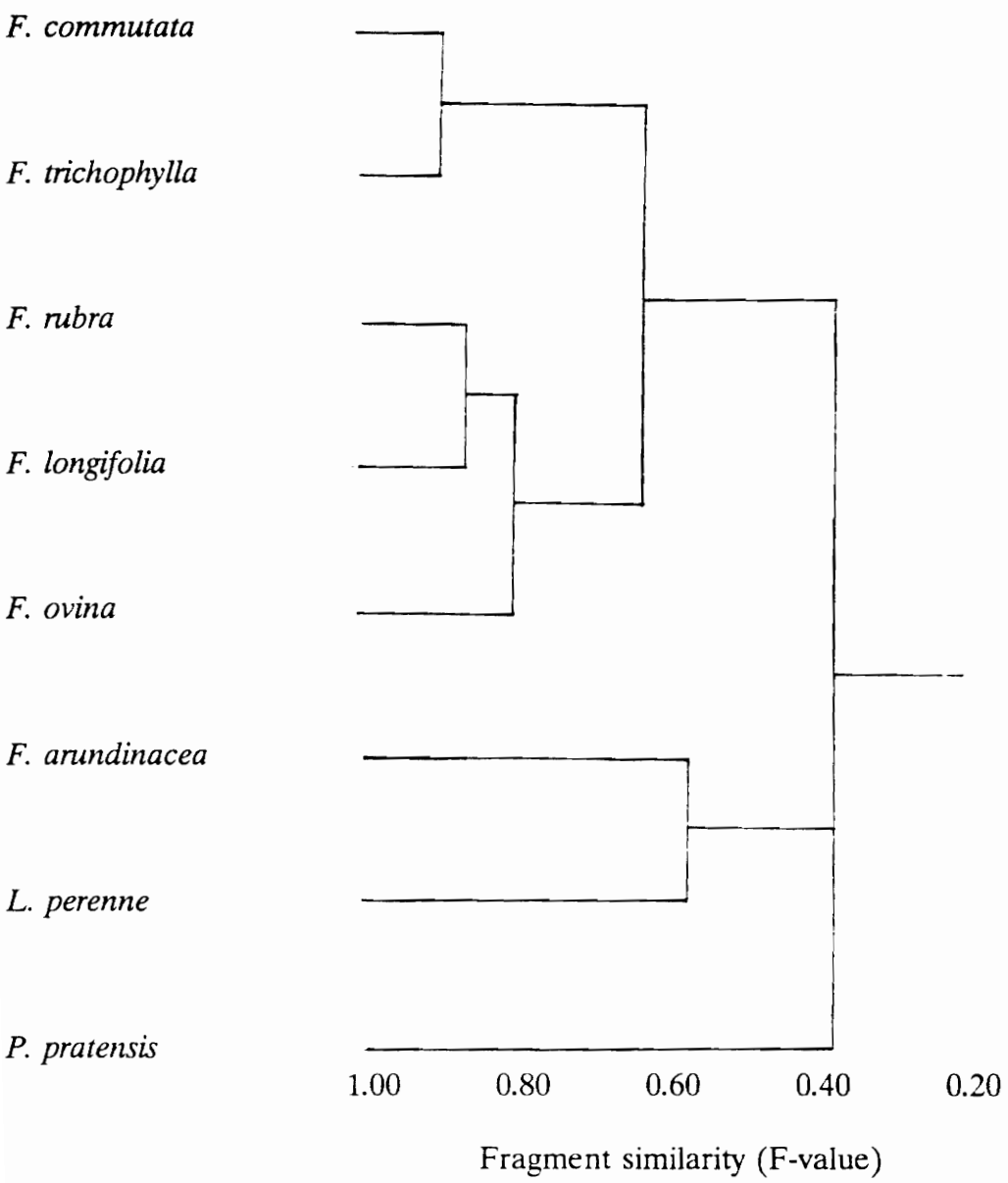

Fig. 5. A dendrogram showing the phylogenetic relationships among eight turfgrasses in *Festuca*, *Lolium*, and *Poa* genera by mtDNA RFLP analysis.

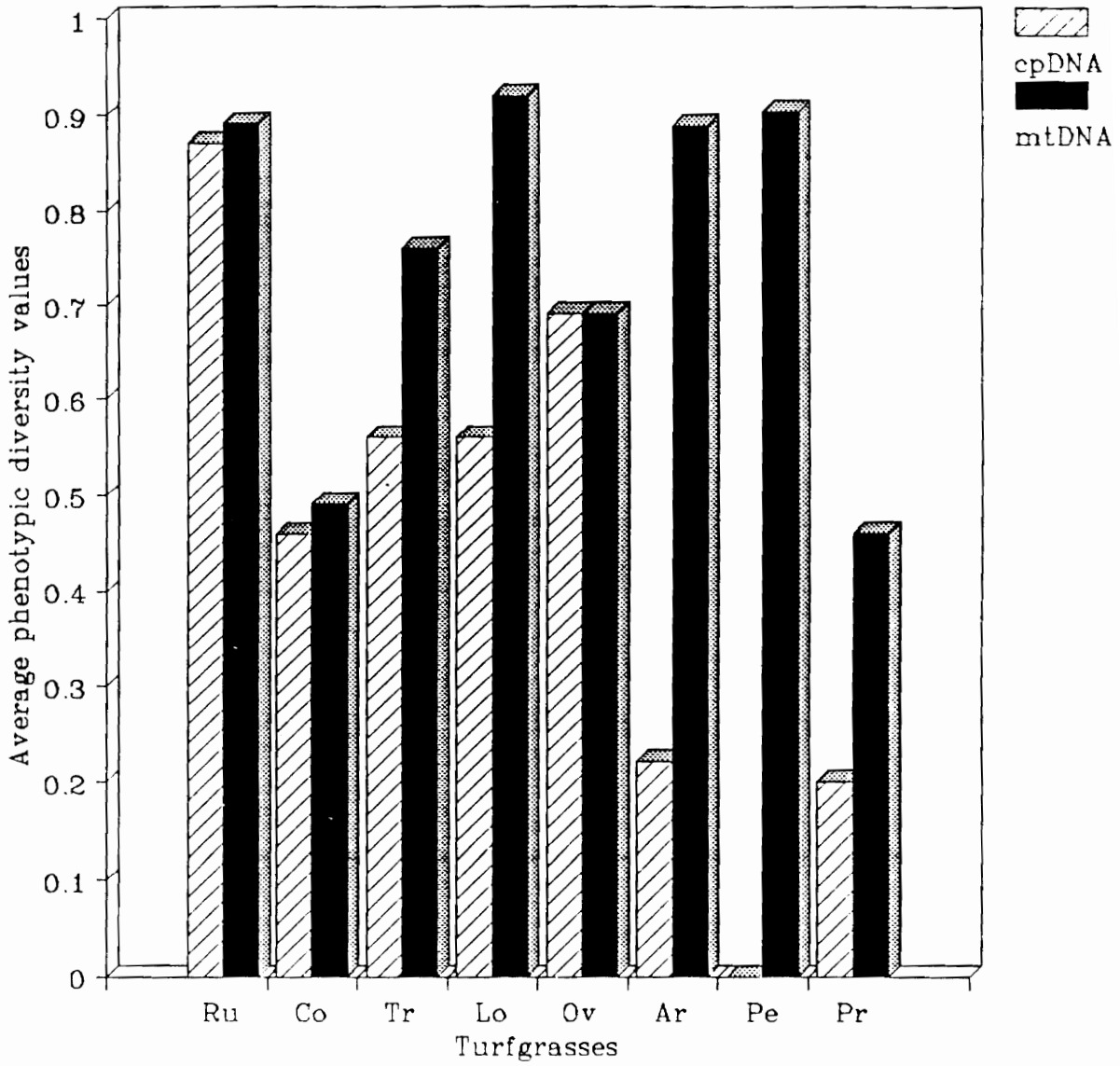

Fig. 6. Comparison of cpDNA and mtDNA phenotypic diversity. Ru=*F. rubra*, Tr=*F. trichophylla*, Co=*F. commutata*, Lo=*F. longifolia*, Ov=*F. ovina*, Ar=*F. arundinacea*, Pe=*L. perenne*, and Pr=*P. pratensis*.

for mtDNA than for cpDNA in *F. rubra, F. commutata, F. trichophylla, F. arundinacea, L. perenne*, and *P. pratensis* (Fig. 7). The percentage of haplotypes was calculated by dividing the number of haplotypes in each species or subspecies by the total number of cultivars in each species or subspecies. For example, the percentage of haplotypes observed in *F. arundinacea* was six times greater for mtDNA than for cpDNA (Fig. 7).

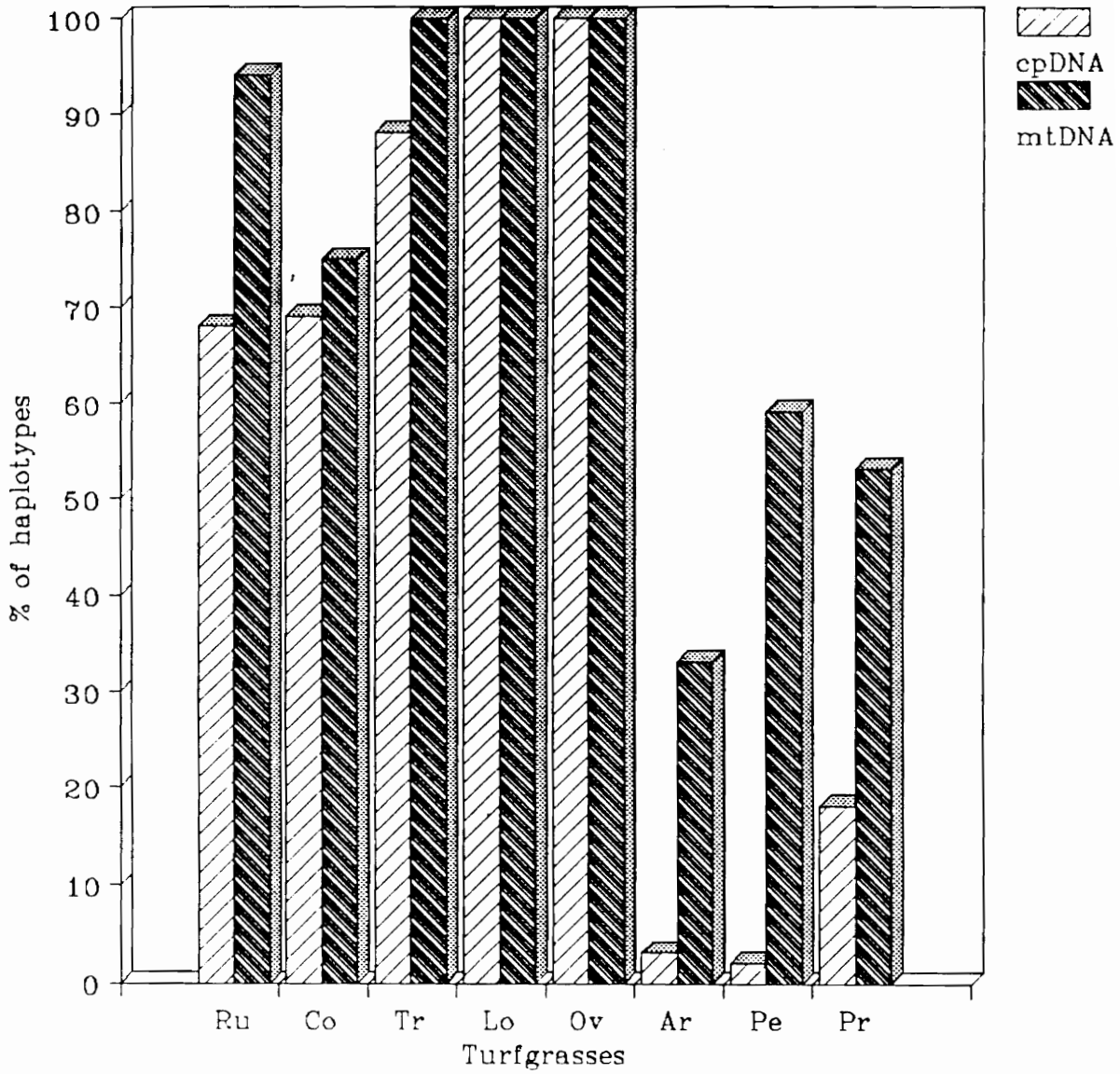

Fig. 7. Comparison of the percentage of haplotypes detected for cpDNA and mtDNA. Ru=*F. rubra*, Tr=*F. trichophylla*, Co=*F. commutata*, Lo=*F. longifolia*, Ov=*F. ovina*, Ar=*F. arundinacea*, Pe=*L. perenne*, and Pr=*P. pratensis*.

Discussion

Results from analysis of mtDNA indicate that the majority of the observed polymorphisms most likely could be attributed to insertion/deletion events. This agrees with results from several other studies on plant mtDNA evolution (Dong and Wagner 1993; Strauss *et al.* 1993; Hooglander *et al.* 1993; Palmer *et al.* 1985a; Bowman *et al.* 1983). Similar results were found in our analysis of cpDNA. In contrast, point mutations have been reported to be the most common cause of variation in the chloroplast genomes of *Linum* (Coates and Cullis 1987), *Heuchera micrantha* (Soltis *et al.* 1989), *Sorghum bicolor* (Duvall and Doebley (1990), and *Zea perennis* (Doebley 1989).

The levels of polymorphism detected by the 19 mtDNA clones of this study were not uniform. Some of the clones (e. g., B376, pQT12, H465, H454, Pst24, 7, B342, and B30) detected a large number of polymorphisms in most taxa, whereas several others (e. g., PYT2-4, Tyr, PFC5-2, Prol, and H469) detected considerably lower levels of variation. In addition, as expected, the detection of RFLPs were also affected by restriction enzymes used in this study. As an example, among the 50 cultivars of *L. perenne*, polymorphism was not revealed when DNA was digested with *Hind* III and probed with the clone H454. In contrast, five different hybridization patterns were detected when the same DNA was digested with *Xba* I and hybridized with the same clone (Table 2). In general, the *Xba* I digests of mtDNA samples revealed the highest polymorphism, followed by *Bam* HI, *Eco* RI, and *Hind* III. A similar result was observed from the study of cpDNA. This suggests that the enzyme *Xba* I is best suited for RFLP analysis for the set of clones and the turfgrasses examined in this study. Thus, careful consideration should be given in selecting proper clone/enzyme combinations from a given experiment or species.

Results from this study suggest that mtDNA is useful as a molecular marker for species and subspecies identification. Estimation of genetic diversity and

germplasm identification based on mtDNA analysis can provide useful information in facilitating the conservation of germplasm and the development of breeding programs. Such applications include the use of mtDNA as a molecular marker to classify the large number of germplasm collections into different groups based on genetic similarities. Such classification should aid in elimination of duplicate accessions in gene banks.

Comparisons of mtDNA variation among turfgrasses

Substantial variation was observed in all six members of the *Festuca* genus. The highest degree of intraspecific mtDNA diversity was detected in *F. longifolia*. In contrast, the lowest was in *F. commutata*, which had only about half of the variability of *F. longifolia*. The highest frequency of polymorphic probe/enzyme combinations was found in *F. rubra*, and the lowest was observed in *F. arundinacea*. These results indicate that a wide range of intraspecific mtDNA variation exists in *Festuca*. In addition, cultivar identification is possible within species and subspecies (i.e., *F. longifolia* and *F. trichophylla*) when combining all polymorphic probe/enzyme combinations. Although only 14% of the total probe/enzyme combinations could detect polymorphisms in *L. perenne*, a relatively high mtDNA diversity value was found among 50 cultivars. Similarly, Xu *et al.* (1992) reported a high level of nuclear DNA variation in this species . An intermediate level of intraspecific mtDNA variation was observed in *P. pratensis*. This result is similar to the observation of Wu *et al.* (1984) who detected variation in this species using isozyme markers.

Species relationships based on mtDNA data

Extensive cytoplasmic polymorphisms among the eight turfgrasses were revealed by RFLP analysis. Each of the eight turfgrasses studied can be distinguished easily on the basis of differences in their DNA fragment banding patterns. For example, the single combination of H465/*Eco* RI detected eight different phenotypes,

identifying all eight turfgrasses. These results indicate that mtDNA should be a useful genetic marker for species identification in turfgrasses. In order to elucidate the relationships among turfgrasses, the most common mtDNA phenotypes were selected to represent each turfgrass. Comparative analysis of shared fragments allows for the elucidation of species relationships between different turfgrasses. The two subspecies with the highest F-value (0.91) were *F. trichophylla* and *F. commutata*. A higher proportion (or F-value) of shared fragment banding patterns of mtDNA among these subspecies is indicative of a greater degree of cytoplasmic genetic homogeneity, and suggests that they are closely related and may have a common origin. Observations from analysis of mtDNA were similar to those obtained for cpDNA as described in Chapter I. The mtDNA-based branching pattern in Fig. 5 shows the three major groups in these eight turfgrasses.

Based on morphological and cytological studies, *F. arundinacea* and *L. perenne* have traditionally been clustered in two different taxonomic genera. However, our results based on the analysis of mtDNA indicate that *F. arundinacea* is more closely related to *L. perenne* than to the other turfgrasses studied. For example, from Table 17, it can be seen that the F-value between *F. arundinacea* and *L. perenne* is 0.43, while F-values range from 0.25 to 0.30 between *F. arundinacea* and others. Similarly, the analysis of cpDNA, discussed in Chapter I, indicates that there is a close relationship between *F. arundinacea* and *L. perenne*. These results are in agreement with the observation of Webster and Buckner (1970) that *F. arundinacea* would hybridize with *L. perenne* but not with the other species.

P. pratensis has an index of relatedness in the range of 0.26 to 0.30 with the other seven turfgrasses, which infers that a rather low degree of relationship exists. A similar observation has been made from cpDNA analysis of the same materials (Chapter I). Apparently, this species is apomictic and has a different cytoplasmic origin from the others, which may be related to its restrictive reproductive mechanism. However, in a study on RFLPs of rDNA for only two accessions,

Ohmura *et al.* (1993) pointed out that *P. pratensis* was closely related to the *Festuca* genus.

Interpretations for mtDNA versus cpDNA polymorphisms

As previously discussed, the degree of polymorphism revealed in the mitochondrial genomes varied greatly from one species to another, yet a higher degree of both intra- and inter-specific variability was generally detected in mtDNA versus cpDNA. Similar conclusions were made in studies on *Zea mays* (Pring and Levings, 1978, Timothy *et al.* 1979), *Lycopersicon* (McLean and Hanson 1986), *Hordeum vulgare* and *H. spontaneum* (Holwerda *et al.* 1986), and *Oryza* (Ishii *et al.* 1993). The variability in mtDNA patterns in all species markedly contrasts with the uniformity observed in cpDNA in *L. perenne*, and the relatively low cpDNA variation in *F. arundinacea* and *P. pratensis*. These findings imply that the mtDNA in these species evolves at a higher rate than cpDNA. These observations contradict the findings of Palmer and Herbon (1988), and Wolfe *et al.* (1987), who reported that the mitochondrial genome in several monocots and dicots evolves at a lower rate than the chloroplast genome. These contradictions may be attributable to differences either in the plant materials or organellar DNA regions studied. For example, we examined RFLPs of the entire chloroplast and mitochondrial genomes of the same turfgrass cultivars for each assay, while Wolfe *et al.* (1987) compared DNA sequence divergence in the coding and non-coding regions of chloroplast and mitochondrial genes of wheat, maize, and tobacco.

Previous reports (McLean and Hanson 1986, Holwerda *et al.* 1986, and Ishii *et al.* 1993) and results from this study have revealed that the mitochondrial genome possesses a higher level of diversity than the chloroplast genome as a result of possible structural variation including rearrangements that occur frequently as length mutations (Sederoff and Levings 1981; Sederoff 1987), rather than point mutations, that occur at a single site within noncoding regions. The larger genome of

mitochondria versus chloroplasts may also be correlated with an increase in the level of observed polymorphisms.

Summary

The study presented here represents the first mtDNA RFLP analysis with a large-scale sampling of cultivated turfgrass species to investigate the level of intra- and inter-specific variation. These results indicate that there is extensive intraspecific polymorphism for mtDNA in *F. longifolia, L. perenne, F. rubra, F. arundinacea, and F. trichophylla*, and an intermediate level of intraspecific variation within *F. ovina, F. commutata*, and *P. pratensis*. In addition, comparison of phenotypic diversities revealed a higher level of variation for mtDNA than for cpDNA. These results have demonstrated that mtDNA should be a useful molecular marker for species and cultivar identification in turfgrasses. For some species, when combining all polymorphic probe/enzyme combinations for both cpDNA and mtDNA, a higher percentage of haplotypes could be revealed than for either cpDNA or mtDNA alone. Although the degree of diversity detected in mitochondrial genomes is higher than in chloroplast genomes, the phylogenetic relationships among the eight turfgrasses examined appear to be congruent based on the analysis of both cpDNA and mtDNA.

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Chapter III

Introduction

The nuclear ribosomal DNA (rDNA), the genes coding ribosomal RNA, occurs universally in all organisms. The genes for rRNA represent one class of repetitive DNA families. The rRNA genes (rDNA) are repeated DNA sequences arranged in tandem arrays at one or a few chromosomal locations. The typical physical structure of a higher plant ribosomal DNA repeat unit contains two major regions: the transcribed (coding) region and the intergenic spacer (IGS) or non-coding region as shown in Fig. 1. The transcribed region includes 18S, 5.8S, and 26S ribosomal RNA sequences. The IGS region of each repeat unit contains an array of tandemly repeated sequences referred to as subrepeats.

The levels, patterns, distribution, and genetics of rDNA variation have been extensively studied by RFLP analysis in plants (Oono and Sugiura 1980; Appels *et al.* 1980; Saghai Maroof *et al.* 1984, 1990; Yakura *et al.* 1984; Doyle and Beachy 1985; Flavell *et al.* 1986; Schaal and Learn 1988; Allard *et al.* 1990; Govindaraju and Cullis 1992; Lanaud *et al.* 1992). Based on these studies, analysis of rDNA can be very useful in obtaining information on evolution and population biology, and for inferring the phylogenetic relationships between species. Extensive variability in the copy number of rDNA repeat units has been found in plants (Appels and Honeycutt 1986; Rogers and Bendich 1987; Zhang *et al.* 1990). The rDNA copy number has been shown to vary by as much as four-fold in *Pisum sativum* (Cullis and Davies 1975), and by ten-fold in inbred lines of maize (Rivin *et al.* 1986). However, all regions of the rDNA do not evolve at the same rate. The rDNA coding regions tend to be highly conserved evolutionarily, while the IGS region evolves rapidly in both sequence and length in closely related species (Appels and Dvorak, 1982). In general, there is no measurable variation in the length of coding regions of the rDNA repeat units in

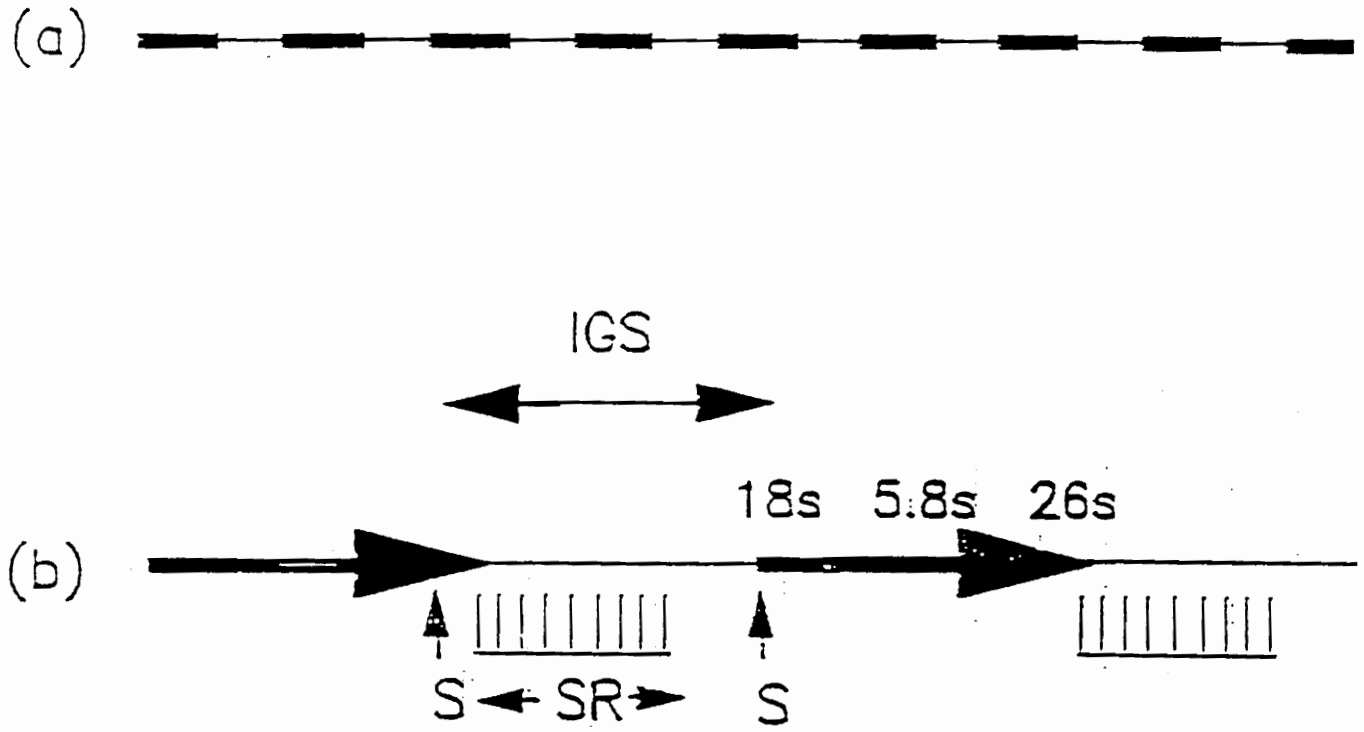

Fig. 1. Ribosomal RNA gene (rDNA) organization (Saghai Maroof *et al.* 1984). a) Tandem arrays of transcribed genes separated by intergenic spacer (IGS) regions. b) Expands two complete repeat units and illustrates the tandem subrepeats in each IGS; SR refers to subrepeat; "S" and the vertical arrows indicate the *Sst* I cleavage sites.

plants. For example, sequencing of the rice, maize, and soybean 18S genes have shown these cistrons to be 1,812, 1,809, and 1,807 bp in length, respectively (Tanaka *et al.* 1980; Messing *et al.* 1984; Eckenrode *et al.* 1985). However, extensive intraspecific and interspecific polymorphisms in the IGS region have been detected in many different plants (Appels and Honeycutt 1986). Spacer length (SL) heterogeneity which appears to be due to variability in copy number of subrepeats in the middle region of the IGS has been observed among species and populations (Appels and Dvorak, 1982; Saghai Maroof *et al.* 1984). Polymorphism in SL appears to be common in plants. Results from several studies indicate that length of the subrepeats varies by no more than a few base pairs within most plant species (Appels and Dvorak, 1982; Saghai Maroof *et al.* 1984). In other words, the sequences of subrepeats within the IGS are substantially conserved within a species. The size of subrepeats can range from 100 to 350 base pairs for different plant species. For example, in barley, wheat, and broad bean, subrepeats are 115, 130, and 325 bp, respectively (Appels and Dvorak, 1982; Saghai Maroof *et al.* 1984; Yakura *et al.* 1984). Variation in length of the IGS region is due to variation in the number of tandem copies of subrepeats. Unequal crossing-over presumably is a primary factor contributing to the variation in copy number of both subrepeats and rDNA repeat units (Flavell 1986).

It is not completely clear whether there is any adaptive significance for variation in copy number of the subrepeat and rDNA repeat units. Adaptedness appeared to be less closely correlated with rDNA copy number than with number of subrepeats in the IGS region in wild barley (Allard *et al.* 1990). In addition, phenotypic and adaptive effects correlated with non-coding sequence variation have been reported by Templeton *et al.* (1989). Correlation of rDNA polymorphism with environmental factors has been observed by Flavell *et al.* (1986). Similar conclusions were made from a study on pitch pine populations by Govindaraju *et al.* (1992), who reported that low copy numbers of the subrepeats was closely correlated with

susceptibility to environmental stress.

The IGS sequences are thought to play a special role in rDNA expression. They may act as functional regulatory elements which act as enhancers of transcription by protein binding (Reeder 1984; Rogers and Bendich 1987). Transcription rates can be increased either by increasing gene copy number or by enhancing the transcription process itself. For example, the subrepeats within the IGS of *Xenopus* possess enhancer activity, which increases the level of transcription from downstream coding regions irrespective of their orientation (Reeder 1984). In addition, the presence of higher copy numbers of subrepeats in the IGS region may favor greater rDNA transcriptional activity (Cluster *et al.* 1987).

Variation in the IGS region is an extremely sensitive measure of rDNA variability in both plants and animals. Because the IGS region evolves much more rapidly than the coding regions, polymorphisms of IGS may be used as molecular markers for estimating genetic distances, for cultivar identification, pedigree analysis, and also for evolutionary studies. Furthermore, the biological significance of variation in copy number, spacer length, and sequences in the IGS within species is still not completely understood and needs further study (Clegg 1989).

In Chapters One and Two, the cytoplasmic diversity of cpDNA and mtDNA in eight turfgrass species and subspecies has already been discussed. In this Chapter, the variation in nuclear rDNA detected in these eight turfgrasses will be discussed.

Materials and Methods

Plant materials

Plant materials used in this survey were the same as those described in Chapter I.

Source of rDNA clone

Source of rDNA clone

One clone, pTA71, a complete rDNA repeat unit from a nuclear genomic library of wheat (Gerlach and Bedrook 1979) was used in this study. This clone was selected because it is highly homologous to turfgrass rDNA and revealed variation in our preliminary studies. A diagram of plant ribosomal DNA organization is shown in Fig. 1. The plasmid containing the wheat rDNA insert was amplified as described in Chapter I.

DNA preparation, RFLP assays, and data analysis

The approaches used for DNA isolation, Southern blot hybridization, autoradiography, and data analysis were the same as described in Chapter I (Materials and Methods section). Restriction enzymes used in this investigation included: *Sst* I, and/or *Kpn* I, *Bam* HI for detecting rDNA variation in the IGS region, and *Sst* I, *Kpn* I, *Bam* HI, *Hind* III, and *Xba* I for coding regions. Digested DNA fragments were size fractionated by electrophoresis in 1.2% agarose gels with a running buffer (1M Tris, 0.125 M NaAC, 0.01 M EDTA, pH 8.1) at 60 milliamps for 72 hours. The size of rDNA fragments was measured using the method described by Southern (1979).

Results

No variation was observed in the rDNA coding regions from any of the species when digested with *Sst* I, *Kpn* I, *Bam* HI, *Hind* III, or *Xba* I. However, variation was found in the IGS region for these turfgrasses examined.

Intraspecific rDNA variation in genus Festuca

rDNA variation in *F. rubra*-- Intraspecific polymorphisms in the rDNA among

16 cultivars in this subspecies were surveyed. Extensive polymorphisms were detected with each of the three enzymes in the IGS region. Phenotypic frequencies and diversity values are summarized in Table 1. From this table, it can be seen that six, seven, and eight different spacer length (SL) phenotypes (banding patterns) were revealed with *Bam* HI, *Kpn* I, and *Sst* I, respectively. The highest diversity value was detected with the *Sst* I digests, and the lowest with those of *Bam* HI. Most of the phenotypes were three or four-banded. An example of an autoradiogram with the *Sst* I digest is shown in panel A (lanes 1-6) of Fig. 2. Six rDNA SL phenotypes were detected and the size of the SL varied from 5.8 to 6.7 kb. When combining data from the three probe/enzyme combinations, 15 unique phenotypes were observed. Cultivars Franklin and Jasper could not be distinguished from each other.

rDNA variation in *F. commutata*--RFLPs of rDNA among 17 cultivars in this subspecies were examined with the same enzymes as in *F. rubra*. Substantial variation was revealed in the SL of the IGS region. The rDNA SL phenotypes, frequencies, and phenotypic diversity values are summarized in Table 1. In the case of the *Sst* I digests, 64% of the cultivars were five-banded (phenotype a) , 18% of the cultivars were four-banded (phenotype b), and 6% of the cultivars had two to three bands (phenotype c, d, e). Therefore, the distribution of the rDNA SL phenotypes in this subspecies was not uniform. Similar observations were found with both *Kpn* I and *Bam* HI digests. An example of intraspecific polymorphism detected with the *Sst* I digests is shown in panel C (lanes 1-5) of Fig. 2. Five SL phenotypes were disclosed in the IGS region and the size of SL varied from 5.8 to 7.2 kb. When combining data from the three probe/enzyme combinations, 12 unique phenotypes were observed. Five cultivars (Shadow, Atlanta, Wilma, Enjoy, and Lolet) could not be distinguished from each other.

rDNA variation in *F. trichophylla*--Many rDNA polymorphisms were observed in the SL of the IGS region in the nine cultivars studied. As shown in Table 1, four, six, and seven different SL phenotypes were identified with the three enzymes

Table 1. rDNA SL phenotypes, frequencies, and phenotypic diversity values detected for six turfgrasses in *Festuca* using clone pTA71 and three enzymes.

Enzyme	Phenotypes & their frequencies								Phenotypic diversity
	a	b	c	d	e	f	g	h	
F. rubra									
Sst I	0.13	0.13	0.44	0.06	0.06	0.06	0.06	0.06	1.731
Kpn I	0.06	0.38	0.06	0.32	0.06	0.06	0.06		1.577
Bam HI	0.19	0.38	0.06	0.25	0.06	0.06			1.538
									Average 1.615
F. commutata									
Sst I	0.64	0.18	0.06	0.06	0.06				1.200
Kpn I	0.64	0.06	0.12	0.12	0.06				1.132
Bam HI	0.12	0.06	0.70	0.06	0.06				1.011
									Average 1.114
F. trichophylla									
Sst I	0.14	0.14	0.15	0.15	0.14	0.14	0.14		2.069
Kpn I	0.29	0.15	0.14	0.14	0.14	0.14			1.744
Bam HI	0.33	0.17	0.17	0.33					1.334
									Average 1.716
F. longifolia									
Sst I	0.33	0.17	0.17	0.17	0.16				1.562
Kpn I	0.25	0.25	0.25	0.25					1.388
Bam HI	0.25	0.25	0.50						1.040
									Average 1.330
F. ovina									
Sst I	0.50	0.50							0.692
Kpn I	0.50	0.50							0.692
Bam HI	0.50	0.50							0.692
									Average 0.692
F. arundinacea									
Sst I	0.04	0.33	0.04	0.13	0.13	0.22	0.11		1.731

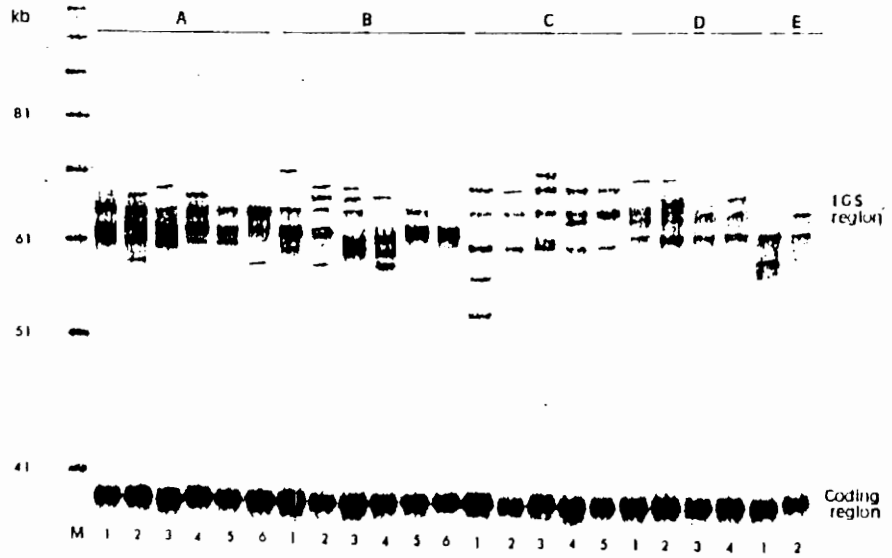

Fig. 2. Autoradiogram of Southern blot showing rDNA SL phenotypes observed in five turfgrasses of fine fescue subgenus when genomic DNA was digested with *Sst* I and probed with clone pTA71. A: lanes 1-6, six phenotypes in *F. rubra*; B: lanes 1-6, six phenotypes in *F. trichophylla*; C: lanes 1-5, five phenotypes in *F. commutata*; D: lanes 1-4, four phenotypes in *F. longifolia*; and E: lanes 1 and 2, two phenotypes in *F. ovina*. Lane M is 1 kb λ marker.

studied. The highest phenotypic diversity value was detected with the *Sst* I enzyme, followed by the *Kpn* I, and *Bam* HI digests. Six of the seven SL phenotypes detected with *Sst* I are shown in panel B (lanes 1-6) of Fig. 2. The majority of the SL varied in size from 5.8 to 6.8 kb. All seven cultivars of this subspecies are distinguishable from each other with the enzyme *Sst* I.

rDNA variation in *F. longifolia*--A total of six cultivars were examined for rDNA variation in this species. Extensive polymorphisms in the SL of the IGS region were detected with all three enzymes. Five, four, and three different SL phenotypes were revealed with the enzymes *Sst* I, *Kpn* I, and *Bam* HI, respectively (Table 1). Most of the phenotypes were four- or five-banded. The range of phenotypic diversity values were from 1.040 to 1.562 with an average of 1.330. Fig. 2, panel D (lanes 1-4) shows an example of an autoradiogram of Southern hybridization with the *Sst* I digests which detected four different SL phenotypes in the IGS region. The majority of the SL varied in size from 5.8 to 6.8 kb. Five of the six cultivars in this species are distinguishable from each other with the enzyme *Sst* I. Cultivars Biljart and Valda could not be distinguished from each other.

rDNA variation in *F. ovina*--Only two cultivars were surveyed in *F. ovina*. Two SL phenotypes were detected in the IGS region with the *Sst* I digests and are shown in panel E (lanes 1 and 2) of Fig. 2. The two cultivars are distinguishable from each other with any of the three enzymes (Table 1).

rDNA SL heterogeneity in the fine fescue subgenus--rDNA SL heterogeneity among 50 cultivars in the fine fescue subgenus including the species and subspecies of *F. rubra*, *F. commutata*, *F. trichophylla*, *F. longifolia*, and *F. ovina* was examined by combining data from assays with *Sst* I. The *Sst* I cleavage of each DNA sample yielded one invariant band (about 3,880 bp) made up primarily of the transcription unit, as well as a series of bands which represents the major portion of the IGS region. The latter bands varied in length as a result of differences in the number of rDNA subrepeats. An extensive length polymorphism in the IGS region was

observed and the fragments ranged in size from 5,066 to 10,258 bp. As shown in Table 2 and Fig. 3, 15 different rDNA SL bands were detected which constitutes a 15-step ladder. As shown in Fig. 3, a total of 13 (A through M) rDNA phenotypes (banding patterns) were observed in the fine fescue subgenus.

rDNA variation in *F. arundinacea*--rDNA intraspecific variability among 46 cultivars in this species were studied with the enzyme *Sst* I. Substantial variation in the IGS region was detected. A total of seven different SL phenotypes were revealed (Table 1). Phenotype b which was three-banded occurred at a relatively high frequency (0.33) among cultivars, followed by phenotype f (0.22), while the other phenotypes had considerably lower frequency. The *Sst* I digests had a phenotypic diversity value of 1.731, and SL varied in size from 4.5 to 5.5 kb.

Intraspecific rDNA variation in genus Lolium

A total of 44 cultivars in this species were surveyed with the enzyme *Sst* I. One invariant (3880 bp) and a set of length variable fragments were generated from each rDNA repeat unit by this enzyme. As shown in Fig. 4, ten different SL phenotypes (A through J) were observed in the IGS region. Four of the ten phenotypes were six-banded (E, F, G, and H), one phenotype was four-banded (D), one (A) was nine-banded, and two phenotypes were seven- and eight-banded (B, J, C, and I), respectively. In addition, a 13-step ladder was detected with the *Sst* I digests (Fig. 4). The rDNA fragment sizes in the IGS region are listed in Table 3. The shortest and longest rDNA SL variants (bands) were 5,973 and 11,533 bp, respectively. Frequencies of the ten different SL phenotypes in the IGS region are shown in Table 4. The phenotypic diversity value was 1.773. Phenotypes e and h occurred at relatively high frequencies among cultivars, while the other phenotypes were relatively rare (Table 4).

Table 2. Calculated relative sizes for *Sst* I rDNA fragments of five turfgrasses in the fine fescue rDNA.

rDNA variant (band) #	Calculated size (bp)
1	5,066[a]
2	5,317
3	5,444
4	5,698
5	5,824
6	6,077
7	6,205
8	6,587
9	6,839
10	6,965
11	7,217
12	7,469
13	8,612
14	9,621
15	10,258

[a] Calculations described by Southern (1979).

Fig. 3. Autoradiogram of Southern blot showing 13 (A-M) SL phenotypes and 15 (1-15) steps revealed in five turfgrasses in fine fescue subgenus when genomic DNA was digested with *Sst* I and probed with clone pTA71. Lane M is 1 kb λ marker.

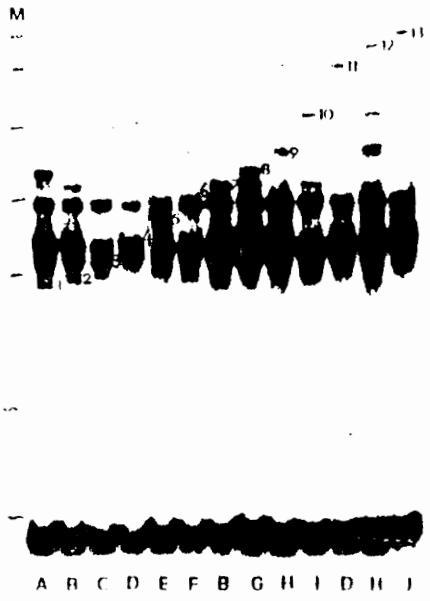

Fig. 4. Autoradiogram of Southern blot showing 10 (A-J) SI, phenotypes and 13 (1-13) steps revealed in *L. perenne* when genomic DNA was digested with *Sst* I and probed with clone pTA71. Lane M is 1 kb λ marker.

Table 3. Calculated relative sizes for *Sst* I rDNA fragments of *L. perenne*.

rDNA variant (band) #	Calculated size (bp)
1	5,973[a]
2	6,084
3	6,306
4	6,529
5	6,863
6	7,085
7	7,417
8	7,640
9	8,085
10	8,863
11	10,310
12	10,978
13	11,533

[a] Calculations described by Southern (1979).

Table 4. rDNA SL phenotypes, frequencies, and phenotypic diversity values detected for the two turfgrass genera in *Lolium*, and *Poa* using clone pTA71 and Sst I enzyme.

Phenotypes & their frequencies										Phenotypic
a	b	c	d	e	f	g	h	i	j	diversity
L. perenne										
0.02	0.05	0.05	0.14	0.23	0.04	0.04	0.39	0.02	0.02	1.773
P. pratensis										
0.09	0.48	0.02	0.11	0.20	0.02	0.04	0.02	0.02		1.712

Intraspecific rDNA variation in genus Poa

rDNA variation among 46 cultivars within this species were examined and nine different SL phenotypes were observed in the IGS region. One of the nine phenotypes was one-banded, six were two-banded, and two were three-banded. As shown in Table 4, phenotype b, which was two-banded, occurred in a predominantly high frequency among the cultivars, while the other phenotypes were considerably lower in frequency. The phenotypic diversity value detected with the *Sst* I digests was 1.712. The SL varied little in size from 5.4 to 5.8 kb.

Interspecific rDNA variation

High variability at the interspecific level in the rDNA IGS region was detected with the enzyme *Sst* I (Table 5). Eight distinct SL phenotypes in the IGS region were detected with the *Sst* I digests, and phenotypic diversity value was 2.076. All eight turfgrasses were distinguishable from each other. In addition, substantial interspecific variation in the coding regions was revealed with four of the five enzymes examined. Phenotypes, frequencies, and estimated phenotypic diversity values are summarized in Table 6. Seven different phenotypes in the coding regions were detected by the *Xba* I enzyme, two by *Bam* HI, and five phenotypes each were detected by *Kpn* I and *Eco* RI. Phenotypic diversity values ranged from 0.664 to 1.903 with an average of 1.385. The frequencies (F-values) of shared fragments among the eight distinct rDNA genomes for the *Bam* HI probe/enzyme combination are shown in Table 7. From this table, it can be seen that the fraction of shared fragments decreased from 1.0 among the three subspecies *F. rubra*, *F. trichophylla* and *F. commutata*, and between species of *F. ovina* and *F. longifolia* to 0.20 between *F. arundinacea* and *L. perenne*. A dendrogram drawn based on the data from Table 7 is shown in Fig. 5. The branching pattern (Fig. 5) shows the three major groups in the eight turfgrasses based on the rDNA analysis. Species *F. arundinacea* and *P. pratensis* were classified in one group, and *L. perenne* was classified in a group by

Table 5. rDNA SL phenotypes, frequencies, and levels of phenotypic diversity detected with *Sst* I digests among eight turfgrasses in *Festuca*, *Lolium*, and *Poa* genera using clone pTA71.

Enzyme	Phenotypes & their frequencies								Phenotypic
	a	b	c	d	e	f	g	h	diversity
Sst I	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	2.076

Table 6. Phenotypes, frequencies, and levels of phenotypic diversity detected in rDNA coding regions for eight turfgrasses in *Festuca*, *Lolium*, and *Poa* genera†.

Enzyme	Phenotypes & their frequencies							Phenotypic diversity
	a	b	c	d	e	f	g	
Kpn I	0.38	0.25	0.13	0.12	0.12			1.487
Bam HI	0.38	0.62						0.664
Eco RI	0.38	0.25	0.13	0.12	0.12			1.487
Xba I	0.13	0.25	0.13	0.13	0.12	0.12	0.12	1.903
							Average	1.385

† Only polymorphic probe/enzyme combinations are listed.

Table 7. Proportion (F-value) of rDNA shared fragments between each pair of the eight turfgrasses for the *Bam* HI probe/enzyme combination.

	Tr	Co	Lo	Ov	Ar	Pe	Pr
Ru*	1.00**	1.00	0.67	0.67	0.29	0.29	0.40
Tr		1.00	0.67	0.67	0.29	0.29	0.40
Co			0.67	0.67	0.29	0.29	0.40
Lo				1.00	0.33	0.33	0.50
Ov					0.33	0.33	0.50
Ar						0.20	0.75
Pe							0.25

* Ru=*F. rubra*, Tr=*F. trichophylla*, Co=*F. commutata*, Lo=*F. longifolia*, Ov=*F. ovina*, Ar=*F. arundinacea*, Pe=*L. perenne*, and Pr=*P. pratensis*.

** Proportion (F-value) of shared fragments between each pair of the eight turfgrasses was calculated by the formula: F-value=2 $N_{xy}/(N_x + N_y)$ (Nei and Li 1979).

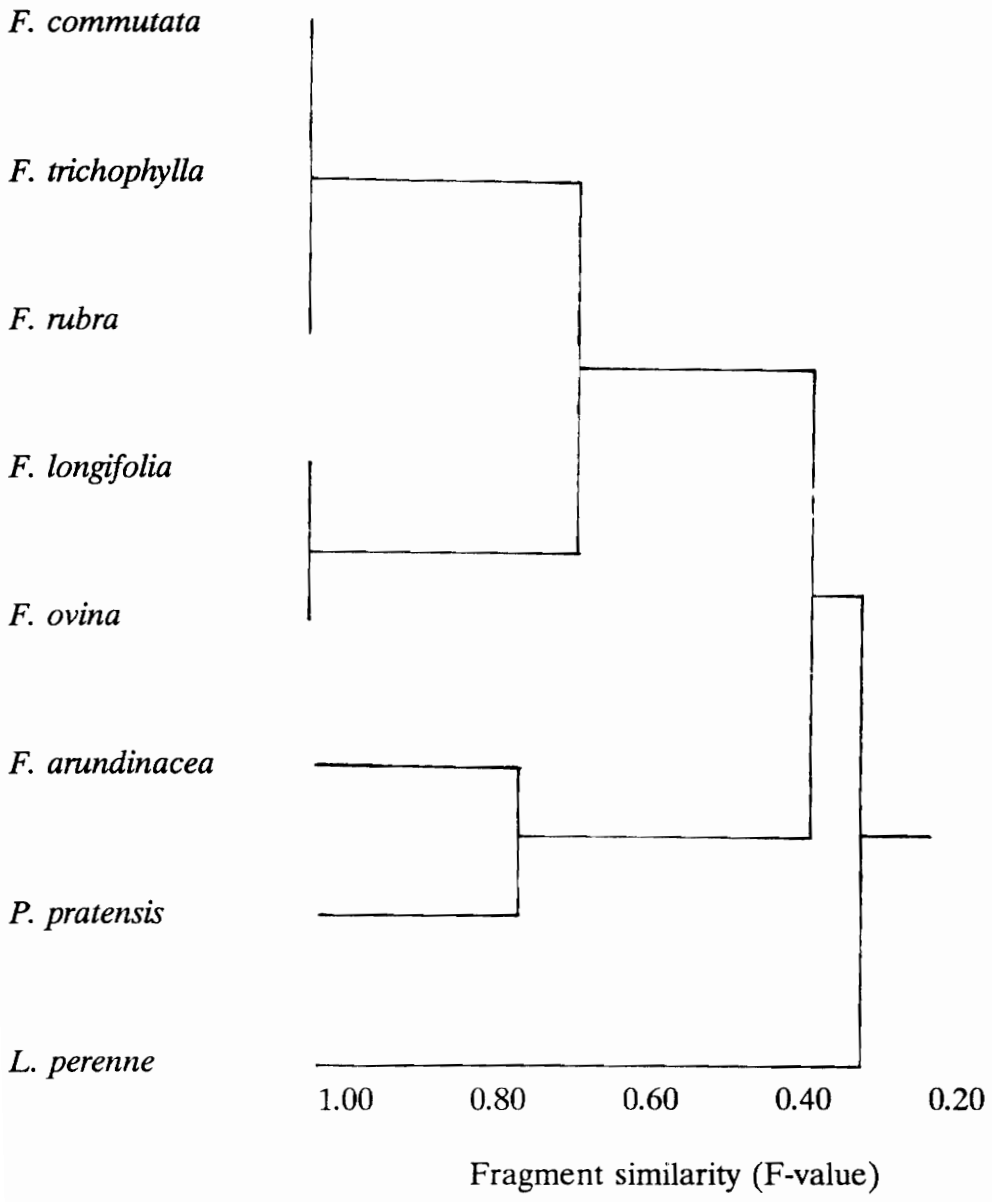

Fig. 5. A dendrogram showing the phylogenetic relationships among eight turfgrasses in *Festuca*, *Lolium*, and *Poa* genera by rDNA RFLP analysis.

itself. The five turfgrasses in fine fescue subgenus were clustered in the same group which could be classified into two different subgroups: one contains three subspecies *F. rubra*, *F. trichophylla* and *F. commutata*, and the other contains two species *F. longifolia* and *F. ovina*.

Polymorphisms detected by rDNA, cpDNA, and mtDNA

Intraspecific variability in each of the eight turfgrasses detected by rDNA (IGS region), mtDNA, and cpDNA is shown in Fig. 6. From this figure, it can be seen that a higher level of variability was found for rDNA than for either mtDNA or cpDNA in all eight turfgrasses except *F. ovina* in which all three DNA types were equal. The rDNA phenotypic diversity value for *F. arundinacea* was twice higher than that of the mtDNA, and about six times higher than that of the cpDNA. The phenotypic diversity value detected for *F. rubra* rDNA was approximately twice that of either cpDNA or mtDNA.

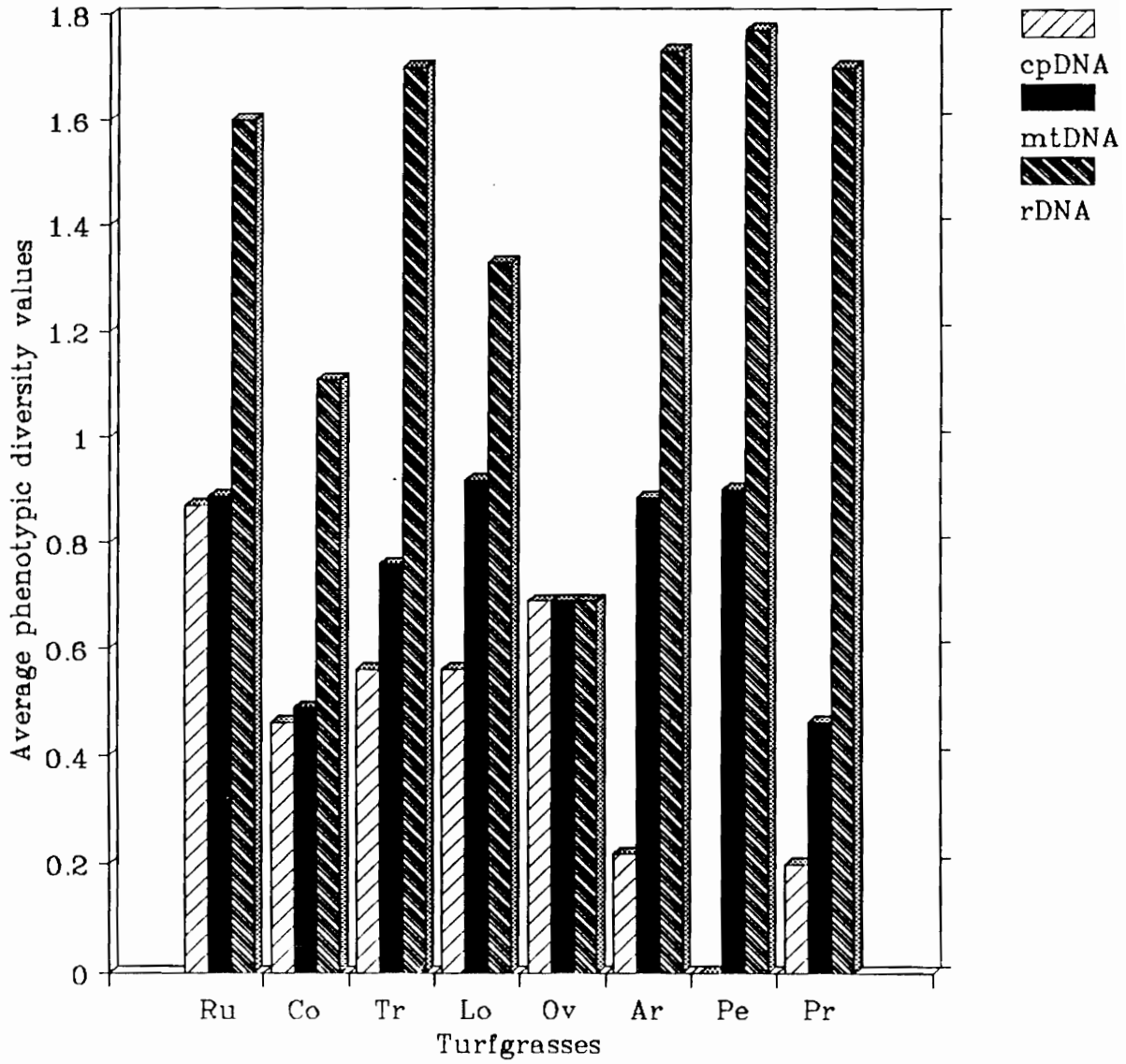

Fig. 6. Comparison of cpDNA, mtDNA, and rDNA (the IGS region) phenotypic diversity. Ru=*F. rubra*, Tr=*F. trichophylla*, Co=*F. commutata*, Lo=*F. longifolia*, Ov=*F. ovina*, Ar=*F. arundinacea*, Pe=*L. perenne*, and Pr=*P. pratensis*.

Discussion

The degree and types of rDNA variation in both the coding and IGS regions within all eight turfgrasses were evaluated. The efficiency of enzymes in detecting RFLPs was variable. The enzyme *Sst* I cleaves each of the several hundred rDNA repeat units twice, once on each side of the IGS region. Thus, *Sst* I cleavage of each DNA sample yields one invariant band (coding region) and a series of length-variable bands reflecting polymorphism due to the variable number of subrepeats of the IGS region. These *Sst* I-generated rDNA fragments have simpler and more distinct patterns than those of *Bam* HI and *Xba* I. Furthermore, *Xba* I fragments consist of both coding and IGS regions and their larger size make it difficult to differentiate the small differences which are due to the number of subrepeats. Therefore, the enzyme *Sst* I is best suited for rDNA analysis of the SL of the IGS region in the turfgrasses studied. However, no difference in the rDNA coding regions was found among the 184 cultivars representing eight turfgrasses when DNA was digested with *Sst* I, whereas as many as seven different phenotypes were detected in the coding region when the DNA was digested with *Xba* I (Table 6). Results from this study indicate that there is genetic variability at the interspecific level in the rDNA coding regions, but no variation was detected at the intraspecific level in these regions. Extensive polymorphisms at both intra- and inter-specific levels were observed in the SL of the rDNA IGS region for each of the eight turfgrasses. Our observations of high variability are similar to studies with other plant species (Appels and Dvorak 1982; Saghai Maroof *et al.* 1984; Gerbi 1985; Appels and Honeycutt 1986). These results indicate that the IGS region is evolving more rapidly than the rDNA coding regions in the turfgrasses examined in this study.

Comparisons of rDNA variation among turfgrasses

Substantial variation in the SL of the IGS region was detected in all eight

turfgrasses. In the case of the *Sst* I digests, a considerably high degree of SL variation was detected in *L. perenne*, *F. arundinacea*, *F. trichophylla*, *P. pratensis*, and *F. rubra*. Ten different SL phenotypes were revealed with the *Sst* I digests in *L. perenne*, and nine SL phenotypes were detected in *P. pratensis*. Seven SL phenotypes were detected in each of *F. trichophylla* and *F. arundinacea*. Similarly, a high variability was observed in the pasture grass species of *F. arundinacea* and *L. perenne* by the analysis of nuclear single copy DNA (Xu *et al.* 1991, 1992), and in *P. pratensis* among most cultivars by isozyme markers (Wu *et al.* 1984; Wehner *et al.* 1976); although, the same cultivars were not used in both studies. The considerably high intraspecific rDNA variation in the IGS region of *L. perenne, F. arundinacea*, and *F. rubra*, is in agreement with the high level of mtDNA polymorphism as discussed in Chapter II. Similarly, extensive rDNA SL heterogeneity in the IGS region also has been reported in other plants such as wheat (Appels and Dvorak 1982), barley (Saghai Maroof *et al.* 1984, 1990), broad bean (Yakura *et al.* 1984), *Phlox divaricata* (Schaal *et al.* 1987), and *Clematis fremontii* (Learn and Schaal 1987). Cultivar identification is possible for some cultivars of all species and subspecies. For example, seven cultivars in the subspecies of *F. trichophylla* are distinguishable from each other with the enzyme *Sst* I. Similar observations were made from the analysis of mtDNA (Chapter II).

Subrepeat length in the rDNA IGS region in the fine fescue and Lolium.

A ladder containing 15 steps (variants or fragments) of different size, was found in our samples in the fine fescue subgenus (Fig. 3). The length of a subrepeat unit is approximately 126 bp. Similarly, 20 steps were identified in the IGS region in a larger sample of barley accessions and the length of a subrepeat unit was 115 bp (Saghai Maroof *et al.* 1984; Allard *et al.* 1990). Eighteen steps within 243 accessions of cultivated and wild rice species have been observed (Sano and Sano 1990). The length differences of rDNA repeats often result from insertion or deletion events of

subrepeats located in the IGS region. Such variation has been reported in animals (Long and Dawid 1980) and plants (Appels and Dvorak 1982; Saghai Maroof *et al.* 1984; Yakura *et al.* 1984). This could also be a reason for length heterogeneity in the rDNA of turfgrass species studied here. From Table 2, it can be seen that the shortest rDNA SL variant is 5,066 bp, while the longest contains 10,258 bp. Of the 50 fine fescue cultivars, most of them had five or six SL variants in addition to the conserved coding region fragment of 3,880 bp. It should be pointed out that the series of 15 steps observed from the 50 samples constitute an incomplete ladder. Based on the DNA fragment band patterns (i.e., positions) and the measurement in the sizes, a complete step ladder in this species should contain 40 steps. Therefore, it is not surprising that some fragments (steps) were not observed in our samples of 50 cultivars. For example, a fragment (step) between steps 1 and 2, 3 and 4, 5 and 6, 8 and 9, 10 and 11, 11 and 12 was not identified.

In *Lolium*, relatively high intraspecific variation in the SL of the IGS regions was detected with *Sst* I. A 13-step ladder was observed in our samples of this species (Fig. 4). The length of a subrepeat unit is approximately 111 bp. An incomplete step ladder was observed, since only 44 samples were examined. For example, a fragment was missing between: steps 2 and 3, 3 and 4, 5 and 6 (Table 3 and Fig. 4). It is expected that a complete step ladder would be obtained with an increase in sample size in this species.

The subrepeat sizes are 126 and 111 bp in length in the fine fescue genus and *Lolium*, respectively. Variation in the numbers of these subrepeats results in rDNA polymorphism and could serve as genetic markers useful for the study of plant diversity and evolution. Such preliminary studies are also important in molecular biology, since the subrepeats contain sequences implicated in polymerase binding, and may be associated with transcriptional activity in some organisms and may play a role in gene expression (Reeder *et al.* 1984).

Species phylogenetic relationships based on rDNA data

The phylogenetic relationships of the eight turfgrasses were elucidated by the same method as used in the analysis of cpDNA and mtDNA. Results from the analysis of rDNA (Table 7, Fig 5) suggest that three subspecies *F. rubra*, *F. trichophylla* and *F. commutata* in the fine fescue subgroup are closely related to each other and may have common origin. Similarly, *F. ovina* appears to be closely related to *F. longifolia*. These results are in agreement with traditional classification (Turgeon 1985). However, different results were obtained from the analysis of cpDNA and mtDNA as discussed in Chapters I and II, and also from the analysis of reproductive biological data (Schmit *et al.* 1974). The latter results suggest that *F. rubra* is more closely related to the species *F. ovina* and *F. longifolia* than to the other subspecies (*F. trichophylla* and *F. commutata)*.

From Table 7 and Fig. 5, it also can be seen that the F-value between *L. perenne* and the other seven turfgrasses ranged from 0.2 to 0.33. These data indicate that *L. perenne* is not closely related to the other turfgrasses and is clustered in a distinct group. This result is contradictory to those obtained from the analysis of organellar DNA as was discussed in Chapter I. Analysis of the organellar DNA indicates that *F. arundinacea* and *L. perenne* are more closely related to each other than to any other taxa. This conclusion is also supported by the observations obtained from pollination and hybridization studies of Webster and Buckner (1970). Their data suggest that *F. arundinacea* is distantly removed from the *Festuca* genus and that its classification should be reconsidered.

F. arundinacea and *P. pratensis* had a relatively high F-value (0.75), which indicates that they are closely related to one another. Ohmura et al (1993) reached a similar conclusion based on rDNA analysis of two accessions of these same species. These rDNA results do not agree with the observations from the analysis based on the organellar DNA polymorphisms which have already been discussed in Chapters I and II. Similar discordance in results, obtained with nuclear versus cytoplasmic

markers, have already been reported for several organisms such as *Drosophila* (Caccone *et al.*, 1988; DeSalle and Giddings, 1986), rice (Sano and Sano 1989) and sugarcane (Glaszmann *et al.* 1989, 1990). In the present study the discordant conclusions from the two classes of markers may be explained by the following: 1) classifications are based on three different genomes which have different modes of inheritance (uniparental vs biparental); 2) rDNA (the IGS region) evolves faster than other sequences such as cpDNA and mtDNA; and 3) nuclear genome is represented only by one rDNA clone. Thus, the use of limited probe/enzyme combinations may not reflect a complete picture of the genomic differences.

Comparison of variability detected by rDNA, cpDNA, and mtDNA

The highest degree of intraspecific variability was detected by rDNA (IGS region), and the least by cpDNA (Fig. 6). These findings indicate that the rDNA in these turfgrasses evolves at a higher rate than either cpDNA or mtDNA, and that mtDNA evolves at a faster rate than cpDNA (Chapter II). The variability in the rDNA pattern markedly contrasts with the uniformity observed in the cpDNA in *L. perenne*. Similarly, a higher level of rDNA polymorphism was detected in *F. arundinacea* and *P. pratensis* compared to the relatively low cpDNA variation . The lower diversity value observed for cpDNA was expected, since chloroplast genomes of higher plants display a remarkable constancy in size, structure, and organization. It is generally believed that DNA sequences in coding regions are highly conserved in evolution, compared to those of non-coding (spacer) regions (Sederoff 1987). Thus, the high proportion of coding sequences in chloroplast genomes may explain why they are highly conserved in evolution. This is in contrast to the nuclear and mitochondrial genomes, which contain large and variable amounts of non-coding sequences. Variation in these genomes may be the result of structural changes including rearrangements that occur frequently as length mutations at a single site within non-coding regions (Sederoff *et al.* 1981; Sederoff 1984, 1987). In addition,

larger genome size in the mitochondrial and nuclear genomes is also correlated with an increase in variability. A high level of diversity, therefore, suggests a rapid rate of evolution for rDNA in turfgrass, in contrast with the chloroplast genome which has less divergence. Similar conclusions have been made in studies recently reported for rice (Ishii *et al.* 1993).

Summary

Intra- and inter-specific variation of both the IGS and coding regions of rDNA for eight turfgrasses has been assessed. The degree, types, and distribution of rDNA and species relationships have been discussed. The levels of variability detected in rDNA, mtDNA, and cpDNA were also compared. These results indicate that substantial genetic variability at the interspecific level has been detected in the rDNA coding regions, but no polymorphism at the intraspecific level was observed in these regions. However, extensive diversity both within and between species and subspecies was detected in the rDNA SL for the same genetic materials. Results from this study have demonstrated that the IGS region is evolving more rapidly than the rDNA coding regions in the turfgrasses examined in this study. In addition, comparisons of genetic variability observed in three different genomes indicate that the highest level of variation was detected by rDNA for the IGS region, followed by mtDNA, and cpDNA.

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Summary

In this study, RFLPs of cpDNA, mtDNA, and rDNA were used to estimate and compare the extent of diversity among the organellar and nuclear genomes, and to infer species relationships. Eight cultivated turfgrass species and subspecies were used in this study including: *Festuca rubra*, *F. commutata*, *F. trichophylla*, *F. longifolia*, *F. ovina*, *F. arundinacea*, *Lolium perenne*, and *Poa pratensis*. Genomic DNA from tissue samples of 208 cultivars representing the eight turfgrasses was digested with each of four restriction enzymes: *Hind* III, *Bam* HI, *Eco* RI, and *Xba* I, and probed with a set of ten barley cpDNA clones, nineteen wheat mtDNA clones, and one wheat rDNA clone. RFLPs of the three different genomes were detected through Southern blot and hybridization.

The degree, type, and distribution of diversity detected within and between these species and subspecies were compared by RFLP analysis. Relative phenotypic diversity in the cytoplasmic and nuclear genomes was evaluated using Shannon's information statistic (Bowman *et al.* 1971). Genetic similarities used for computing species relationships were based on the proportion of shared RFLP fragments according to Nei and Li (1979).

Extensive nuclear and cytoplasmic DNA variation was detected with RFLP markers in the cultivated turfgrass species. DNA fragment patterns detected by autoradiography in the three genomes indicate that most of the observed variation most likely could be attributable to insertion/deletion events. Restriction enzymes differed in their ability to detect RFLPs among particular cultivars and genomes.

In general, there was substantial intraspecific cpDNA variation in most members of the *Festuca* genus; while a relatively low level of diversity existed within *P. pratensis*, and no diversity was found within *L. perenne*. Results from the analysis of mtDNA indicate that extensive intraspecific polymorphisms exist in most members of the *Festuca* genus and *L. perenne*, and an intermediate level of variation is present

within *P. pratensis*. Results from the analysis of rDNA indicate that variability exists in the rDNA coding region at the interspecific level, but no polymorphism was detected at the intraspecific level. However, extensive diversity both within and between species and subspecies was detected in the rDNA SL for the same materials. Comparisons of phenotypic diversity values revealed a higher degree of variability for nuclear than for organellar genomes. Results also indicate that mtDNA in these species evolves at a slower rate than rDNA but at a faster rate than cpDNA. The variability in the rDNA pattern markedly contrasts with the uniformity observed in the cpDNA in *L. perenne*. On the other hand, there was substantial interspecific variation detected in all three genomes. Analysis of species relationships based on data from mtDNA is in accordance with those from cpDNA. Results from these analyses indicated that *F. arundinacea* and *L. perenne* are closely related to each other, and that *F. rubra* is more closely related to *F. longifolia* and *F. ovina* than to the other subspecies. This coincidence reflects the co-evolution between cpDNA and mtDNA genomes through maternal inheritance. On the other hand, analysis of species relationships based on data from rDNA is in agreement with those from the traditional classification. This is the first study where the data from three genomes have simultaneously been used to address genetic variation and species relationships in plants.

A total of 13 distinct rDNA SL phenotypes were identified among 50 cultivars in the fine fescue subgenus, and a ladder containing 15 steps of different size were detected with *Sst* I digests. The length of a subrepeat in the IGS region measured in the fine fescues is about 126 bp. Similarly, a total of 13 steps in the IGS region were found among 44 cultivars in *L. perenne*. The length of a subrepeat in the IGS region in this species is approximately 111 bp. The subrepeat length (i. e., subrepeat number) located within the spacer region between the rDNA genes could serve as genetic markers of rDNA alleles and is potentially useful for the studies of plant diversity and evolution.

Extensive rDNA and cytoplasmic genetic diversity detected by these markers should provide useful information for the evaluation and conservation of germplasm and in the development of breeding programs in turfgrasses. For example, the molecular markers of this study can be used for variety identification purposes, and as criteria in germplasm collection as well as in the elimination of duplicates in gene bank samples. Chloroplast and mitochondrial DNA markers can also be used in the generation of populations with a higher level of cytoplasmic diversity and in determining the mode of inheritance (paternal/maternal) of organellar DNA. Further investigations using other types of DNA markers such as single-copy sequences, microsatellites as well as DNA sequencing would be desirable in resolving the phylogenetic relationships among the eight turfgrasses of this study.

VITA

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