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Paternal inheritance of mitochondria and chloroplasts in *Festuca pratensis-Lolium perenne* intergeneric hybrids

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Abstract. Organelle inheritance in intergeneric hybrids of *Festuca pratensis* and *Lolium perenne* was investigated by restriction enzyme and Southern blot analyses of chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA). All F_1 hybrids exhibited maternal inheritance of both cpDNA and mtDNA. However, examination of backcross hybrids, obtained by backcrossing the intergeneric F_1 hybrids to *L. perenne*, indicated that both uniparental maternal organelle inheritance and uniparental paternal organelle inheritance can occur in different backcross hybrids.

Key words: Organelle inheritance – Chloroplast DNA – Mitochondrial DNA – *Festuca pratensis-Lolium perenne* hybrids

Introduction

In the plant kingdom, mitochondria are generally inherited from the maternal parent only (Hanson and Conde 1985), although some degree of paternal transmission of both chloroplasts (reviewed by Smith 1988) and mitochondria (Soliman et al. 1987; Neale et al. 1989; Erickson and Kemble 1990) has been reported. Organelle inheritance patterns have been studied using both genetic and molecular approaches. Genetic analyses of mitochondrial inheritance have relied principally on the mutant mitochondrial phenotype of cytoplasmic male sterility (CMS) (Rhoades 1931; Edwardson and Warmke 1967), although in these studies analysis of cytoplasmic inheritance was not the primary objective and, consequently, the numbers of progeny examined were small. In addition, the rarity of CMS mutants and the paucity of other non-lethal mutant mitochondrial phenotypes has resulted in few detailed genetic analyses of mitochondrial inheritance in plants. In contrast, the widespread availability of chloroplast mutations such as those leading to albinism, herbicide and antibiotic resistance (Kirk and Tilney-Bassett 1978; Souza-Machada et al. 1978) has resulted in a far more comprehensive view of chloroplast inheritance patterns.

However, in recent years improved methods for both isolating and analysing organellar DNA have provided a more comprehensive picture of mitochondrial inheritance in plants. These data generally corroborate earlier findings of strict maternal mitochondrial inheritance in angiosperms (Conde et al. 1979; Vedel et al. 1978, 1981; Timothy et al. 1979; Samoilov et al. 1986; Breiman 1987), even in species that exhibit paternal chloroplast inheritance. For example, Schumann and Hancock (1989) reported that mitochondrial DNA (mtDNA) was inherited maternally in *Medicago* despite the occurrence of chloroplasts of paternal origin within the same plants. However, in gymnosperms paternal mtDNA inheritance is commonplace (Neale et al. 1989, 1991; Wagner et al. 1991). Ultrastructural studies suggest that in some species of gymnosperms abundant organelles of paternal origin are present in the zygotic cytoplasm and that, furthermore, mechanisms for the selective elimination of maternally inherited organelles may occur during early development (Chesnoy 1987). In contrast, it has been suggested that in angiosperms, depending on the particular species, organelles are either not transmitted through the pollen parent at all, or are inactivated in the early zygote (Connett 1987). Nevertheless, the observation of

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exceptions to paternal inheritance of mtDNA in gymnosperms (Neale and Sederoff 1989; Wagner et al. 1991) and maternal inheritance of mitochondria in angiosperms indicate that the organelle exclusion and inactivation mechanisms are not absolute. Examples of paternal inheritance of mtDNA in angiosperms include an early report by Brennicke and Schwemmle (1984) of an interspecific *Oenothera* hybrid that contained mtDNA identical to that of the paternal parent. Biparental inheritance of organelle DNA, in which individual F_1 hybrids contained mtDNA and chloroplast DNA (cpDNA) restriction fragments diagnostic for each parent has been described in *Hordeum-Secale* F_1 intergeneric hybrids by Soliman et al. (1987). More recently, a larger scale study by Erickson and Kemble (1990) demonstrated that 10% of progeny from an intraspecific *Brassica napus* cross inherited mtDNA biparentally, although cpDNA was inherited from the maternal parent only.

In this report, we trace the inheritance of organelles in hybrids of *Festuca pratensis* and *Lolium perenne* and find evidence for uniparental maternal and uniparental paternal inheritance of both mitochondria and chloroplasts.

Materials and methods

Plant material

The source of commercial varieties of *F. pratensis* and *L. perenne* and material from a CMS ryegrass breeding programme including backcross hybrid generations 1–7 (BC_1–BC_7) and a CMS ryegrass line have been described elsewhere (Kiang et al. 1993); Connolly and Wright-Turner 1984). The CMS line used in the present analysis was 9B290, a ninth generations CMS backcross hybrid. A further set of F_1 and backcross 1 (B_1) hybrids were produced from the pair crosses outlined in Table 1. Viable progeny were recovered either by zygotic embryo culture or the culture of naturally set seeds (see below). Leaf material obtained from single plants of the parental species, F_1 hybrids and backcross hybrids was used in all investigations involving Southern blot analysis. Pooled etiolated seedlings of *F. pratensis*, *L. perenne* and CMS *L. perenne* were used for the extraction of purified organelle DNAs.

Table 1. F_1 hybrids (F_1) and backcross generation-1 hybrids (B_1) resulting from intergeneric hybridisation between *F. pratensis* (F_p) and *L. perenne* (L_p)

Parentage Female	Male	Progeny Embryo culture	Seed
F_p var 215	× L_p var Talbot	F_1m; F_1o; F_1t; F_1v	F_1a
L_p var Talbot	× F_p var 215	–	F_1Rc
F_p var Remko	× L_p var Francis	F_1n; F_1s	–
F_p var 215	× L_p var Gremie	F_1b; F_1h; F_1k; F_1u; F_1v; F_1y	–
F_1h	× L_p var Talbot	B_1h	–
F_1m	× L_p var Talbot	B_1m	–

Zygotic embryo culture

Ten days following pollination developing embryos were aseptically excised from ovules and cultured on 0.8% agar containing Gamborg's B5 medium (Gamborg et al. 1968) and 3% sucrose at 21 °C in the dark. Following germination the plantlets were transferred to an illuminated growth room with a photoperiod regime of 16 h (day) 8 h (night). Two weeks later the plantlets were transferred to sterile glass tubes containing 0.8% agar, one-fifth dilution of Gamborg's medium and 0.5% sucrose. On reaching a height of 10 cm the plantlets were re-planted in vermiculite and watered with a one-fifth dilution of Gamborg's B5 medium; after approximately 4 weeks they were potted in soil and transferred to an unheated greenhouse.

Seed culture

Seed were obtained from a number of hybrids without the requirement for embryo culture. These were surface sterilised and then germinated and cultured as described for zygotic embryo culture.

DNA probes

Several cloned DNA probes were used to investigate the inheritance of nuclear DNA, cpDNA and mtDNA in the hybrids. pML17 contains a *Hin*dIII-defined rRNA gene repeat unit of 10.2 kb from melon, cloned into pACYC 184 (Kavanagh and Timmis 1988). Cloned maize mitochondrial genes including *coxI* (pBN6601; Isaac et al. 1985b), *coxII* (pZME1; Fox and Leaver 1981), and *atpA* (pZMH5; Isaac et al. 1985a) were kindly provided by Prof. C. J. Leaver, University of Oxford. pLCP1 contains a 7.5-kb *Bam*HI cpDNA fragment from *L. perenne* cloned into pUC19.

DNA extractions

MtDNA was isolated using a modified differential centrifugation technique as described in Kiang et al. (1993). Chloroplasts were isolated from a fraction pelleted at 1000 *g* during the preparation of a purified mitochondrial pellet. Chloroplast lysis and purification of cpDNA was carried out as described for mitochondria and mtDNA. Total cellular DNA was extracted from 0.2–1 *g* of leaf tissue. The tissue was homogenised as rapidly as possible in 1.5 ml of extraction buffer(100 m*M* NaCl, 100 m*M* Tris-HCl pH 8.0, 2% SDS, 65 m*M* 2-mercaptoethanol) and was immediately deproteinised by the addition of 1 ml of Tris-saturated phenol followed by vigorous homogenisation. The resulting slurry was clarified by centrifugation at 12 000 *g* and the aqueous phase re-extracted 3 times with phenol-chloroform. RNase A was added to a final concentration of 100 ug/ml and digestion allowed to proceed for 20 min at 42 °C. Following two further phenol-chloroform extractions DNA was precipitated in two volumes of ethanol. The precipitate was collected by centrifugation, washed in 70% ethanol, air dried and then resuspended in 300 ul TE (10 m*M* Tris-HCl pH 8.0, 1 m*M* EDTA).

DNA analyses

Restriction enzyme digestions were carried out according to the manufacturer's instructions (Boehringer Mannheim), except that organellar DNAs were digested for 4 h and total cellular DNAs for 8 h Restriction digests were electrophoresed through 0.5% (mtDNA) 0.7% (cpDNA) and 0.8% (total cellular DNA) agarose gels in 1 × TBE buffer at 4 V/cm for 12–20 h. Probe labelling and Southern hybridisations were carried out as previously decribed (Kiang et al. 1993).

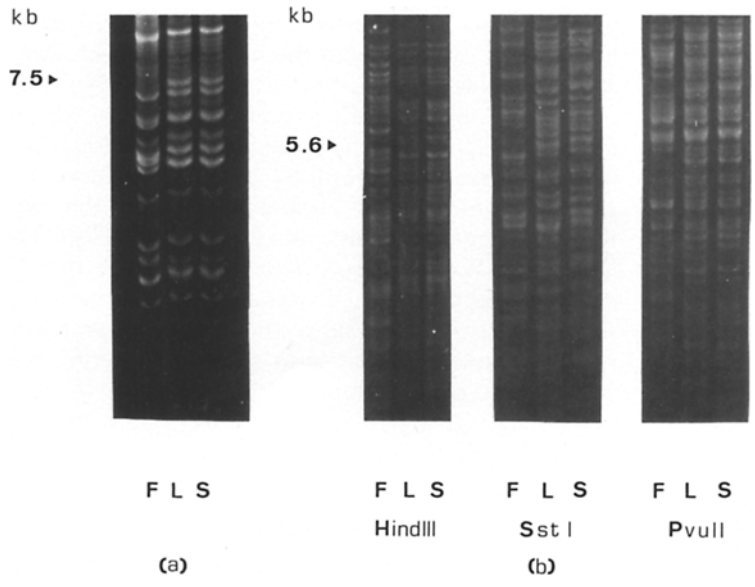

Fig. 1a, b. Restriction enzyme digests of purified organellar DNAs from *F. pratensis* (*F*), *L. perenne* (*L*) and CMS *L. perenne* line 9B290 (*S*) **a** *Bam*HI digests of cpDNA; **b** *Hin*dIII, *Sst*I and *Pvu*II digests of mtDNA

Results

A CMS line contains cpDNA and mtDNA similar to that of the paternal parent

The CMS *L. perenne* lines described by Connolly and Wright-Turner (1984) were initially derived from an intergeneric cross between *F. pratensis* as the female parent and *L. perenne* as the pollen parent. The *L. perenne* nuclear background was subsequently reintroduced by backcrossing the F_1 intergeneric hybrids (as the maternal parent) to the original pollen parent (*L. perenne*) for nine generations.

Consequently, we had expected that the CMS line 9B290 would contain organellar DNAs identical to those of *F. pratensis.* However, restriction enzyme digestion profiles of cpDNA and mtDNA from CMS9B290 were either identical or almost identical, respectively, to those of original paternal parent *L. perenne* and differed greatly from those of the maternal parent *F. pratensis* (Fig. 1). For example, *Bam*HI-generated cpDNA restriction fragment profiles clearly showed that a 7.5-kb fragment diagnostic for *L. perenne* was also present in CMS9B290 (Fig. 1a). Likewise, mtDNA restriction fragment profiles of the CMS line were almost identical to those of fertile *L. perenne* but were quite different from those of *F. pratensis* (Fig. 1b). The single most obvious difference between the mtDNAs of the fertile parental *L. perenne* and CM9B290 was the absence of a 5.6-kb *Hin*d-III fragment in mtDNA of the CMS line.

Backcross hybrids contain mtDNA similar to that of the derived CMS line

Insufficient material from the early backcross generations necessitated the identification of the mtDNA types by probing total cellular DNA digests with a number of cloned mitochondrial gene probes. In order to determine whether the early backcross hybrids generated during the CMS *L. perenne* breeding programme of Connolly and Wright-Turner (1984) also contained *L. perenne*-like cytoplasm, total cellular DNA from single plants representing backcross generation hybrids 1, 3, 5 and 7 (denoted BC_1, BC_3, BC_5 and BC_7) was digested with either *Sal*I or *Hin*dIII and subjected to a Southern blot analysis using a cloned maize cytochrome oxidase subunit II (*coxII*) gene as the probe (Fig. 2). This analysis showed that *coxII* sequences were located on a 6.0-kb *Sal*I fragment and a

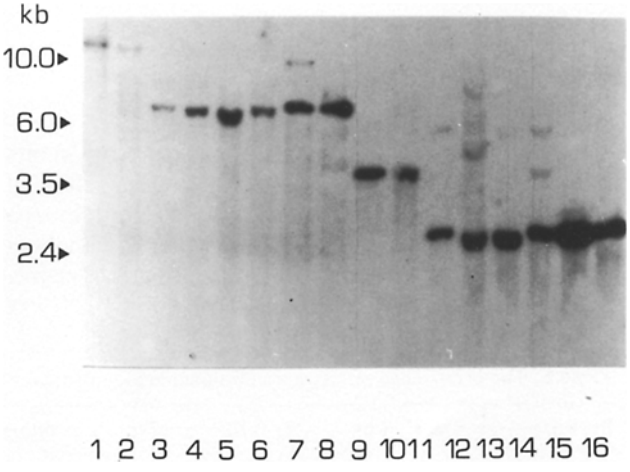

Fig. 2. Southern blot analysis of total cellular DNA from BC hybrids and parental species digested with *Sal*I (*lanes 1–8*) or *Hin*dIII (*lanes 9–16*) and hybridised with the *coxII* probe, pZME1. *Lanes 1, 2, 9, 10 F. pratensis, lanes 3–6* and *11–14* backcross generations 1, 3, 5, 7, respectively, *lanes 7, 8, 15, 16 L. perenne*

2.4-kb *Hind* III fragment in fertile *L. perenne* and all of the backcross hybrids examined, whereas in *F. pratensis*, *cox*II sequences were located on a 10.0-kb *Sal*I fragment and a 3.5-kb *Hind* III fragment. This suggests that by the first backcross generation, *L. perenne*-like mtDNA had already been established in the progenitor CMS line. Unfortunately, since material from the original F_1 generation was no longer available, it was not possible to pinpoint more precisely the timing of the paternal transfer event.

Production of new F_1 and backcross generation-1 (B_1) hybrids

We decided to investigate more fully the occurrence of paternal cytoplasmic transmission by reconstructing the original intergeneric cross between *F. pratensis* and *L. perenne* described by Connolly and Wright-Turner (1984). Details of the parental varieties used in pair crosses and the resulting progeny recovered are presented in Table 1. The production of hybrids by both embryo culture and seed culture was attempted for all pair crosses. However, embryo culture proved by far the most successful method, providing progeny from every cross except the reciprocal intergeneric cross. In all, 13 F_1 hybrids (F_1a, F_1b, F_1h, F_1k, F_1m, F_1n, F_1o, F_1s, F_1t, F_1u, F_1v, F_1w and F_1y) and two B_1 hybrids (B_1h and B_1m) were recovered. A single F_1 hybrid from the reciprocal intergeneric cross (F_1Rc) in which *F. pratensis* was the paternal parent was also obtained. Pollen viability in the hybrids was typically low as indicated by the collapsed, elliptical morphology and light staining of pollen grains compared with the darkly staining round pollen grains from fertile *L. perenne* (data not shown).

Detection of parental nuclear DNA sequences in the hybrids

The truly hybrid nature of the F_1 progeny plants obtained from the intergeneric cross, regardless of whether they were recovered from seed or embryo culture, was demonstrated by Southern blot analysis (Fig. 3). A nuclear ribosomal RNA gene (rDNA) probe, pML17, from melon (Kavanagh and Timmis 1988) was hybridised to *Sst*I-digested total cellular DNA from both parental species and the hybrids. A4.7-kb fragment diagnostic for *F. pratensis* nuclear DNA was observed in all of the F_1 hybrids as well as a 6.0-kb fragment diagnostic for *L. perenne*. The latter fragment was also present in both B_1 hybrids produced by embryo culture (lanes 11 and 12) and in the two backcross generations BC_3 and BC_7 described by Connolly and Wright-Turner (1984). However, the *F. pratensis*-specific fragment was not retained in either set of backcross plants, suggesting that the *F. pratensis* rDNA complement had been eliminated during the first backcross generation.

Maternal mitochondrial DNA inheritance in the F_1 hybrids

An extensive Southern blot analysis of total cellular DNA samples employing three restriction enzymes (*Eco*RI, *Hind*III and *Bam*HI) and three mitochondrial gene probes, *atp*A(alpha-subunit of the F_1 ATPase), *coxI* and *coxII* (cytochrome oxidase subunits I and II) was carried out in order to determine the pattern of inheritance of mtDNA in the F_1 hybrids. The hybridisation profiles obtained with each combination of probe and restriction digest provided a wealth of markers capable of reliably distinguishing between the mtDNAs of *L. perenne* and *F. pratensis* in total cellular DNA digests (Table 2).

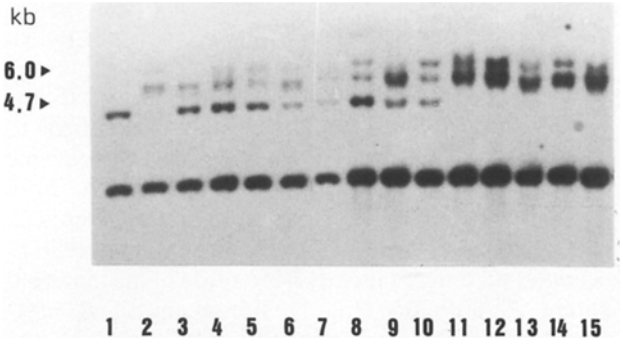

Fig. 3. Southern blot analysis of *Sst*I-digested total cellular DNAs hybridised with the rDNA probe, pML17. *Lane 1 F. pratensis, lanes 2, 15 L. perenne, lane 3* F_1u, *lane 4* F_1m, *lane 5* F_1v, *lane 6* F_1h, *lane 7* F_1h, *lane 8* F_1t, *lane 9* F_1a, *lane 10* F_1Rc, *lane 11* B_1h, *lane 12* B_1m, *lane 13* BC_3, *lane 14* BC_7

Table 2. The occurrence of restriction fragments diagnostic for *F. pratensis* (F_p) and *L. perenne* (L_p) in hybrid plants

Probe/enzyme	F_p (kb)	L_p (kb)	Hybrids examined F_p fragments	L_p fragments
*coxII-Hind*III	3.5	2.4	F_1a; F_1b; F_1h; F_1m; F_1n; F_1o; F_1s; F_1t; F_1u; F_1v; F_1w; F_1y; B_1m	F_1Rc; B_1h
*coxII-Eco*RI	1.9; 2.4	1.8; 2.0	F_1a; F_1h; F_1m; F_1s; F_1t; F_1v;F_1y; B_1m	B_1h
*coxII-Bam*HI	2.5; 10.0	5.2; 6.1	F_1a; F_1h; F_1m; F_1n; F_1o; F_1s;F_1t; B_1m	B_1h
*coxI-Hind*III	1.9	5.0	F_1a; F_1b; F_1h;F_1k; F_1s; F_1u;F_1v; F_1w; B_1m	F_1Rc; B_1h
*atpA-Hind*III	7.2	3.7	F_1h; F_1m; B_1m	B_1h

The hybridisation profiles obtained with the *coxII* probe, for example, and either *Bam*HI-or *Hin*dIII-digested DNAs are presented in Fig. 4. The *coxII* probe identified fragments of 2.5 kb and 10.0 kb in *Bam*HI digests of *F. pratensis* DNA and fragments of 5.2 kb and 6.1 kb in *Bam*HI digests of *L. perenne* DNA. The F_1 hybrids F_1n, F_1s, F_1o and F_1t all contained the 2.5-kb and 10-kb mtDNA restriction fragments diagnostic for *F. pratensis*, the maternal parent, and lacked the *L. perenne* diagnostic fragments (Fig. 4, lanes 1–8). Southern analysis of total cellular DNAs digested with *Hin*dIII from the same plants confirmed these results (Fig. 4, lanes 9–16). The *coxII* probe identified a single fragment of 3.5 kb in *F. pratensis* that was also present in the F_1 hybrids, while the 2.4-kb *L. perenne* fragment was absent from the F_1 hybrids. All of the other F_1 hybrids except the reciprocal hybrid F_1Rc (which, as expected, contained the *L. perenne*-specific mtDNA fragments) contained the *F. pratensis*-specific mtDNA pattern and not that of the paternal parent *L. perenne* (Table 2).

Similar results were obtained using the *coxI* and *atpA* mtDNA probes (Table 2). In all, the combined use of three mtDNA probes and three restiction enzymes identified five informative markers, each of which confirmed the exclusive maternal inheritance of mtDNA in the F_1 generation hybrids.

Paternal mitochondrial DNA inheritance and maternal mitochondrial DNA inheritance in different B_1 hybrids

The inheritance of *coxII*-homologous *Bam*HI mtDNA fragments in the B_1 hybrids B_1m and B_1h, is shown in Fig. 5. *CoxII* sequences were located on fragments of 10.0 kb and 2.5 kb in *F. pratensis* (lane 1) and on fragments of 6.1 kb and 5.2 kb in *L. perenne* (lane 8). Consistent with the results shown in Fig. 4, all of the F_1 hybrids contained the fragment pattern of the maternal parent *F. pratensis*. In addition, B_1m exhibited the 10.0-kb and 2.5-kb fragments diagnostic for *F. pratensis*. However, the *coxII*-homologous sequences of B_1h were located on fragments of 6.1 kb and 5.2 kb diagnostic for the paternal parent *L. perenne*. The data presented in Table 2 extend these observations and confirm the fact that B_1h contained only those restriction fragments that are diagnostic for the paternal parent, while B_1m contained only those fragments diagnostic for the maternal parent.

Chloroplast DNA inheritance in the hybrids

The 7.5-kb *Bam*HI cpDNA restriction fragment which distinguishes *L. perenne* cpDNA from that of *F. pratensis* (Fig. 1, lanes 2 and 3) was cloned into pUC19 to give pLCP1 and used to probe Southern blots of total cellular DNA from *F. pratensis*, *L. perenne* and the F_1 and B_1 hybrids (Fig. 6). The probe detected *Bam*HI fragments of 3.2 kb and 4.3 kb in *F. pratensis* (lane 10) and a fragment of 7.5 kb in *L. perenne* (lane 1). All F_1 hybrids and B_1m contained the 3.2-kb and the 4.3-kb *Bam*HI fragments diagnostic for *F. pratensis* (lanes 3–9), indicating that maternal inheritance of cpDNA has occurred in these plants. However, sequences homologous to pLCP1 in B_1h were located on the *L. perenne* *Bam*HI diagnostic fragment of 7.5 kb (lane 2). Since there was no evidence of either the 4.3-kb or the 3.2-kb *F. pratensis* cpDNA fragments in B_1h, it was concluded that uniparental paternal inheritance of cpDNA had occurred in this backcross hybrid. The faintly hybridizing fragment migrating at approximately 7.5 kb present in the F_1 hybrids and B_1m (lanes 3-9) was probably the result of partial digestion rather than evidence for

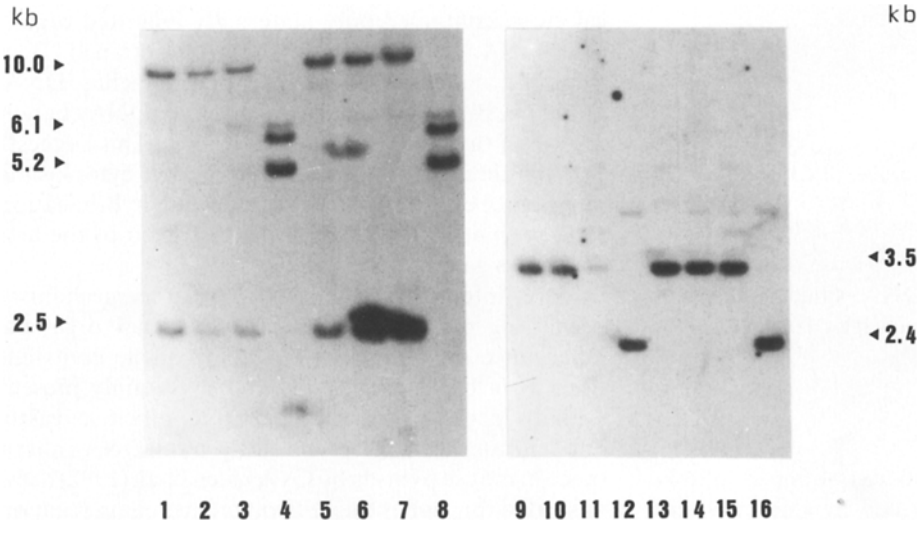

Fig. 4. Southern blot analysis of total cellular DNA from pair cross parents and F_1 progeny digested with *Bam*HI (*lanes 1–8*) or *Hin*dIII (*lanes 9–16*) and hybridised with the *coxII* probe, pZME1. *Lanes 1, 9 F. pratensis* var 'Remko', *lanes 2, 10* F_1n, *lanes 3, 11* F_1s, *lanes 4, 12 L perenne, lanes 5, 13 F. pratensis* var '215', *lanes 6, 14* F_1o, *lanes 7, 15* F_1t, *lanes 8, 16 L. perenne* var 'Talbot'

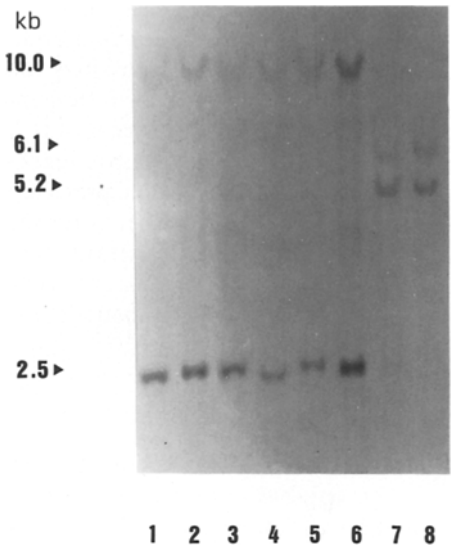

Fig. 5. Southern blot analysis of *Bam*HI-digested total cellular DNAs hybridised with the *coxII* probe, pZME1. *Lane 1 F. pratensis, lane 2* F_1a, *lane 3* F_1t, *lane 4* F_1m , *lane 5* B_1m, *lane 6* F_1h, *lane 7*, B_1h, *lane 8 L. perenne*

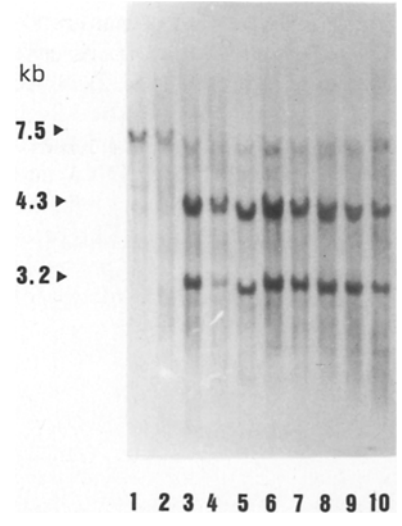

Fig. 6. Southern blot analysis of *Bam*HI-digested total cellular DNA hybridised with the cpDNA probe, pLCP1. *Lane 1, L. perenne; lane 2* B_1h, *lane 3* F_1h, *lane 4* F_1t, *lane 5* F_1s, *lane 6* B_1m, *lane 7* F_1m, *lane 8* F_1a, *lane 9* F_1v, *lane 10 F. pratensis*

the presence of *L. perenne* cpDNA, since it was also present in the *F. pratensis* sample (lane 10).

Discussion

The CMS *L. perenne* line 9B290 was obtained following sexual hybridisations described by Connolly and Wright-Turner (1984) in which the F_1 progeny of an intergeneric cross between *F. pratensis* (female parent) and *L. perenne* (pollen parent) were backcrossed to the pollen parent for nine generations. Genetic and Southern blot analyses of reciprocal crosses between fertile, CMS and restored-CMS lines of *L. perenne* using DNA probes which were unambiguously capable of distinguishing between the fertile and sterile cytoplasms confirmed that the CMS phenotype was transmitted in an exclusively maternal fashion (Kiang et al. 1993).

These observations prompted a comparative investigation of organellar DNA structure in the original parental species and the CMS *L. perenne* line (9B290) by restriction enzyme digestion of purified mitochondrial and chloroplast DNAs. To our surprise the restriction enzyme profiles of mtDNA from the 9B290 were almost identical to those of the paternal parent, *L. perenne*, rather than to those of the maternal parent, *F. pratensis*. Furthermore, analysis of mtDNA in four of the early backcross generation lines (including the first backcross generation) suggested that the paternal inheritance event had occurred either during or prior to construction of the first backcross generation. Thus, the origin of CMS *L. perenne* appears to have involved the inheritance of a structurally modified version of the paternal mitochondrial genome at some stage following the construction of the original intergeneric cross.

In order to confirm the occurrence of paternal cytoplasmic inheritance following *F. pratensis* × *L. perenne* hybridisations and to determine more precisely the timing of the paternal inheritance event, a number of similar intergeneric crosses were reconstructed and a further set of F_1 and backcross-1 (B_1) hybrids obtained. In all, 14 F_1 hybrids originating from four different pair crosses and two B_1 hybrids were recovered. Analysis of the organellar DNA in these hybrids clearly indicated that all of the new F_1 intergeneric hybrids, including the hybrid from the reciprocal cross, contained only maternally inherited organellar DNA. Of the two B_1 hybrids obtained, B_1m exhibited maternal inheritance of organellar DNA. However, B_1h contained cpDNA and mtDNA characteristic of the pollen parent, *L. perenne*. This suggests that the timing of any potential paternal cytoplasmic inheritance events following intergeneric hybridisation in *L. perenne* may be genetically restricted to the first backcross generation.

It is interesting to speculate on the mechanism permitting paternal rather than maternal organelle inheritance in some B_1 hybrids. Organelle contributions from both parents are almost certainly present initially in the newly formed zygote, albeit in vastly different amounts. A recent study of mtDNA inheritance in mouse hybrids by Gyllensten et al. (1992) demonstrated through the use of polymerase chain reaction

(PCR) amplification techniques the persistence of mtDNA of paternal origin in the adult hybrids, although at very low levels. A similar phenomenon has been reported in F_1 hybrids of *Brassica napus* (Erickson and Kemble 1990): mitochondrial DNAs from both parents were readily detectable in 10% of the hybrids, although in this case the paternal complement was estimated to account for up to 84% of the mtDNA in a given hybrid. The initial presence in the zygote of organelles contributed by both parents introduces the possibility that should the normal controls ensuring maternal transmission of organelles be perturbed (by chromosome loss for example), the paternal rather than maternal transmission of organelles might be observed. Indeed, evidence suggesting that a hybrid nuclear genome may confer a selective advantage on particular organellar genotypes has been reported by Schotz (1975) in a study of plastid inheritance in *Oenothera* hybrids.

In the original CMS breeding programme described by Connolly and Wright-Turner (1984), only 6 F_1 (*F. pratensis* × *L. perenne*) intergeneric hybrids were recovered, all of which showed gross chromosomal abnormalities at meiosis and were highly female sterile as well as male sterile. Consequently only 4 of these hybrids were successfully backcrossed to *L. perenne*, and numbers of progeny obtained in the first backcross generation (BC_1) were small. The abnormal nuclear background of the F_1 and early backcross hybrids may have promoted the inheritance of organelles contributed by the paternal rather than the maternal parent. Furthermore, since the paternally inherited mitochondrial genome of the backcross hybrids was not entirely identical to that of fertile *L. perenne* (and most probably confers the CMS trait on the hybrids), it appears that paternal mtDNA inheritance was accompanied by mtDNA rearrangement. A recent study of mtDNA rearrangements in maize (Escote-Carlson et al. 1990) clearly demonstrates that the nuclear genotype can have a profound influence on mtDNA structure. A detailed analysis of mtDNA rearrangments found in the CMS *L. perenne* line 9B290 which correlate with the CMS phenotype will be presented elsewhere (Kiang et al. in preparation).

In the set of hybrids produced for the present study, paternal inheritance of organelles was observed only in a single B_1 hybrid, B_1h, and not in any of the intergeneric F_1 hybrids. Backcrossing the F_1 hybrids to *L. perenne* resulted in the apparently complete elimination of the *F. pratensis* rDNA complement. This may have been accompanied in B_1h by the elimination of loci required for the maintenance of *F. pratensis* organelles, thus confering a selective advantage on the paternal organelle complement. Alternatively, the particular *L. perenne* nuclear genotype of the B_1 hybrids may have affected the incidence of paternal organelle inheritance. In this regard, B_1h differed from B_1m in that two different *L. perenne* varieties, var 'Gremie' and var 'Talbot', were used in the construction of B_1h, whereas var 'Talbot' alone was used in the construction of B_1m (see Table 1).

Simultaneous uniparental paternal inheritance of chloroplasts and mitochondira in plants has only been observed in the gymnosperms *Sequoia* and *Calocedrus* (Neale et al. 1989, 1991) and has not been reported for angiosperms. In angiosperm species where paternal inheritance of chloroplasts has been demonstrated, the mitochondria were inherited in an exclusively maternal fashion (Schumann and Hancock 1989; Schmitz and Kowalik 1986; Schmitz 1988). Moreover, in the *B. napus* hybrids described by Erickson and Kemble (1990) where biparental inheritance of mtDNA was observed, cpDNA was maternally inherited. Biparental inheritance of both organellar genomes has been reported in *Hordeum-Secale* hybrids (Soliman et al. 1987). However, in this case within individual plants, paternal inheritance of one organelle type was always accompanied by maternal inheritance of organelles of the other type and thus differs fundamentally from the *F. pratensis-L. perenne* hybrids discussed in this report. The occurrence of uniparental maternal organelle inheritance in *F. pratensis-L. perenne* F_1 hybrids and either uniparental paternal or uniparental maternal organelle inheritance in different B_1 hybrids is an unusual and interesting observation.

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