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Cytoplasmic male sterility (CMS) in *Lolium perenne* L.: 1. Development of a diagnostic probe for the male-sterile cytoplasm

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Abstract. Analysis of reciprocal crosses between non-restoring fertile genotypes and restored male-sterile genotypes of *Lolium perenne* confirmed the cytoplasmic nature of the sterility trait. This prompted a search for a molecular probe that could be used to distinguish between fertile and cytoplasmic male-sterile (CMS) cytoplasms. We describe the identification and cloning of a 4.5-kb *Bam*HI-*Hin*dIII restriction fragment from the mtDNA of the CMS line. The cloned fragment (pCMS45) failed to hybridise to sequences in the mtDNA of fertile lines and was thus capable of unambiguously distinguishing between fertile and CMS cytoplasms. The use of pCMS45 as a diagnostic probe provided a simple test for positive identification of young non-flowering plants carrying the CMS cytoplasm and also permitted confirmation at the molecular level of the maternal transmission of the CMS trait suggested by the genetic data.

Key words: Diagnostic probe – Mitochondrial DNA (mtDNA) – Cytoplasmic male sterility (CMS) – *Lolium perenne*

Introduction

Cytoplasmic male sterility (CMS) is a maternally inherited trait in which plants fail to produce functional pollen but maintain female fertility. Typically, male fertility is subsequently restored by the introduction of one or more dominant nuclear restorer genes. The CMS trait has been commercially exploited for efficient low-cost production of F_1 hybrid seed in a number of important crop species including maize, sorghum, sunflower and sugar beet (reviewed by Hanson and Conde 1985).

CMS may occur spontaneously or as a consequence of intergeneric, interspecific or intraspecific cross-pollination (Edwardson 1970). Whatever its origin, it is generally accepted that CMS is due to a nuclear-cytoplasmic incompatibility and that different mechanisms are likely to exist in different systems (Hanson 1991). Typically, the consequences of the nuclear-cytoplasmic incompatibility are manifested only during microsporogenesis. Histological investigations have indicated that the CMS phenotype is the result of mitochondrial dysfunction during pollen development (Warmke and Lee 1977).

Recent reports on the molecular genetic nature of CMS have largely focussed on the identification and characterisation of rearrangements of the mitochondrial genome within sterile lines (reviewed by Newton 1988). Although unequivocal evidence is lacking, data from a number of studies strongly suggest that mtDNA rearrangements which arise from both inter- and intramolecular recombination events and which correlate with the CMS phenotype play a causative role in the defect (Hanson 1991). If such rearrangements also alter the restriction enzyme fragment pattern of mtDNA from CMS lines, they can be exploited for diagnostic purposes.

New sources of CMS are continually being evaluated. Until recently, however, CMS cytoplasms were classified solely on the basis of their interactions with nuclear restorer genes. Clearly this lengthy process could be accelerated if new CMS cytoplasms could be distinguished from previous types on the basis of specific alterations in their mitochondrial (mt) DNA restric-

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tion fragment profiles. Correlations between CMS types and the presence or absence of specific mtDNA sequences or restriction fragments have been reported for a number of species. Umbeck and Gengenbach (1983) observed a 6.6-kb *Xho*I mtDNA restriction fragment that was diagnostic for the cms-T cytoplasm of maize. Similarly, Boeshore et al. (1985) identified two *Bgl*II restriction fragments that were diagnostic for CMS in petunia, while an *Nco*I fragment of Ogura radish mtDNA was observed to be diagnostic for CMS in *Brassica* (Bonhomme et al. 1991). In all of these cases, the mtDNA restriction fragments that were diagnostic for the CMS cytoplasm were altered in fertile revertant plants.

The identification of restriction fragment differences in preparations of purified mtDNA from fertile and CMS lines is, however, of limited use in plant breeding programmes where the rapid identification of cytoplasms from small quantities of tissue is required. The use of mtDNA-derived hybridisation probes capable of detecting CMS-specific differences in total DNA, which has been reported for sugar beet (Weihe et al. 1991), may be more appropriate in these circumstances.

In the investigation reported here we confirm the cytoplasmic nature of male sterility in an *L. perenne* line initially derived from an intergeneric cross between *Festuca pratensis* and *L. perenne* (followed by repeated backcrossing to *L. perenne*) (Connolly and Wright-Turner 1984). We also describe the identification of mtDNA sequences that appear to be unique to the male-sterile line. These sequences were cloned in a plasmid vector in order to generate a probe capable of specifically identifying the CMS cytoplasm in young vegetative plants. We have used the CMS-specific probe to follow the inheritance of the sterile cytoplasm in the progeny of reciprocal crosses between *L. perenne* lines carrying either normal or sterile cytoplasms.

Materials and methods

Plant material

Commercial fertile varieties of *L. perenne* and *F. pratensis* were purchased from D. J. Van der Have BV, The Netherlands. All of the CMS plants used in this study were sixth or seventh generation backcross genotypes derived from the original intergeneric hybrid and were phenotypically *Lolium* for all characters other than pollen fertility. It is therefore assumed that most or all of the *Lolium* nuclear genotype was recovered during the backcross programme. Three kinds of genotype were used as follows:

1) CMS lines: male-sterile lines.
2) Maintainer lines: fertility non-restoring genotypes selected from previous backcross generations and used as the pollen parent in reciprocal crosses. These genotypes contain the normal fertile cytoplasm but are homozygous recessive for nuclear fertility restoring genes.
3) CMS-restored genotypes: fertile progeny identified in segregating families obtained by crossing CMS lines with plants taken at random from *Lolium* populations. These genotypes carry the CMS cytoplasm but are fertile because of the presence of dominant nuclear fertility restoring gene(s).

All material used was non-inbred except for the maintainer lines, which were partially inbred. Pair crosses and their reciprocals were carried out under greenhouse conditions. In order to ensure that none of the seed obtained could result from self-pollination, all female parents were emasculated and the flowering heads enclosed in pollen-proof bags.

Assessment of fertility/sterility

All plants were grown under greenhouse conditions and assessed for fertility/sterility on at least two occasions. Classification of male sterility was based on anther appearance and the percentage occurrence of normal pollen grains as described by Connolly and Wright-Turner (1984). In general, sterile plants produced shrivelled, translucent anthers that were nondehiscent over the whole plant. Samples of anthers from fertile and sterile plants were examined microscopically for the presence of normal pollen grains using the I_2-KI staining procedure described by Brooks and Brooks (1967). When we used this procedure, pollen grains from fertile plants stained a dark blue and were uniformly spherical in appearance. In contrast, 80–96% of the pollen grains from male-sterile plants stained lightly, and were uniformly collapsed and elliptical in appearance, suggesting the absence of cytoplasmic contents.

Extraction of mtDNA

Purified mtDNA was isolated from 100 *g* of 7-day-old etiolated seedlings. The tissue was homogenised in 600 ml of extraction buffer (0.44 *M* sucrose, 50 m*M* TRIS-HCl (pH 8.0), 3 m*M* EDTA (pH 8.0), 0.5% BSA, 10 m*M* β-mercaptoethanol) and filtered through four layers of muslin. The filtrate was centrifuged at 5000 *g* to remove chloroplasts, nuclei and cellular debris. Mitochondria were pelleted by centrifugation at 16 000 *g* and resuspended in 10 ml DNAase buffer (0.44 *M* sucrose, 50 m*M* TRIS-HCl (pH 8.0), 10 m*M* $MgCl_2$) and digested with 10 mg DNAase I for 1 h at 4 °C. The mitochondria were re-pelleted twice through a wash buffer [0.6 *M* sucrose, 25 m*M* EDTA (pH 8.0), 50 m*M* TRIS-HCl (pH 8.0)] and lysed in NTE [10 m*M* NaCl, 10 m*M* TRIS-HCl (pH 8.0), 1 m*M* EDTA (pH 8.0)] containing 1% SDS for 15 min at 37 °C. The mitochondrial nucleic acids were then purified by three phenol and phenol-chloroform extractions and precipitated by the addition of two volumes of ethanol. Following centrifugation, the nucleic acids were resuspended in 500 µl NTE, and RNA was removed by incubation with RNAase A (100 µg/ml) at 37 °C for 30 min followed by two further phenol-chloroform extractions and an ethanol precipitation. Where only smaller amounts of tissue were available, mitochondria were isolated as described above except that the DNAase I treatment was omitted. MtDNA extracted in this way was heavily contaminated with nuclear DNA and was therefore analysed by Southern hybridisation techniques.

Isolation of cloned mtDNA sequences

Restriction digests of purified mtDNA were ligated into pUC19 and transformed into competent *E. coli* DH5-alpha using standard procedures (Maniatis et al. 1982). Transformants were colony blotted onto Hybond-N membranes according to the manufacturer's instructions (Amersham Inc) and hybridised with [^{32}P]-labelled DNA probes, as described below.

Southern blot analysis

DNA was digested with restriction enzymes and transferred to Hybond-N according to the manufacturer's instructions (Amersham Inc). Approximately 50 ng of probe DNA was labelled by incorporation of [^{32}P] alpha-dCTP using the random oligomer method of Feinberg and Vogelstein (1983). Southern hybridisations were performed as described in Maniatis et al. (1982) and washed to a stringency of 0.3 × SSC at 65 °C.

Results

Inheritance of male sterility in L. perenne

Differences between reciprocal crosses is a classical test for the presence of a cytoplasmic component in the inheritance of a character (Jinks 1963). By definition, reciprocal tests cannot be performed with male-sterile lines. However, if fertile restored genotypes which contain the CMS cytoplasm and maintainer lines (non-restorers of fertility) which contain the normal fertile cytoplasm are used, reciprocal tests can be made. The segregation pattern of fertile:sterile progeny in reciprocal pair crosses is summarized in Table 1. The differences between reciprocals is striking. In crosses where the female parent was from a maintainer line, all of the progeny were fertile with no dependance on the segregation of nuclear genes; in the reciprocal cross where the female parent was from a CMS-restored line, all of the progeny are predicted to contain the CMS cytoplasm but are fertile or sterile depending on the segregation of a dominant nuclear restorer gene. In the data presented in Table 1 the segregation pattern is in agreeement with a 1:1 ratio over all crosses. These data clearly indicate that a cytoplasmic component is present in the transmission of the sterility trait in *L. perenne*.

Stability of maternal transmission of male sterility

Three CMS-lines and maintainer lines were propagated over three generations. The parent lines in each generation were propagated in pots and grown in

Table 1. Segregation of male-fertile (F) and male-sterile (S) progenies in reciprocal crosses between maintainer ([F] r r) and restored-CMS ([S] R r) lines[a]

	Cross [F] r r × [S] R r		Reciprocal [S] R r × [F] r r		
	F	S	F	S	χ^2 (1:1)
PR146/43 × 7B273/30	36	0	14	13	0.04 ns
PR146/43 × 7B273/17	34	0	14	13	0.04ns
PR146/43 × 6B1648/26	1	0	14	7	2.33 ns
PR146/43 × 6B999/33	14	0	12	8	0.80 ns
SB21/46 × 6B999/28	14	0	19	10	2.79 ns
SB21/46 × 6B999/31	16	0	11	16	0.93 ns
SB21/46 × 7B255/31	No seed		8	13	1.19 ns
Pooled data	115	0	92	80	0.84 ns
Sum $\chi^2_{(7)}$					8.12 ns
Heterogeneity $\chi^2_{(6)}$					7.28 ns

ns, Not significant

[a] A simple genetic model is assumed and symbolised as follows: [F], [S], fertile and sterile cytoplasms, respectively; R, r, dominant and recessive alleles, respectively, of a nuclear fertility restorer gene, controlling male fertility. F, S, Fertile sterile phenotypes, respectively

Table 2. Evaluation of the stability of inheritance of the CMS phenotype over three generations. Samples of up to 60 plants were evaluated for fertility/sterility

Line	Parents			Generation number					
	CMS		Maintainer	1		2		3	
				F	S	F	S	F	S
1	6B1305/5	×	SB21/46	0	60	0	60	0	60
2	6B1016/19	×	SB21/46	0	60	0	60	0	57
3	6B1016/19	×	T119/46	1	59	5	55	9	51

F, Fertile; S, sterile

isolation in pollen-proof chambers in the ratio 2:1 male-sterile:pollinator. Seed was harvested from each line separately and used to establish the next generation. Samples (60 plants) from each of the three generations of male-sterile lines established in this way were grown simultaneously and evaluated for male sterility/fertility as described in the Materials and methods. Results are summarized in Table 2. For lines 1 and 2 there was a complete stability of transmission of male sterility over three generations. In line 3, a small number of fertile genotypes were found that indicated some degree of reversion to fertility.

Identification of a molecular marker specific for the CMS cytoplasm

In order to facilitate the identification of young non-flowering plants carrying the CMS cytoplasm, we investigated the possibility of generating a CMS-specific probe derived from mtDNA sequences. To this end, restriction enzyme fragment profiles of the mtDNAs of both CMS and fertile. *L. perenne* were compared in order to determine whether changes in the structure of the mitochondrial genome correlated with male sterility. Figure 1(a–c) shows that only minor differences were observed between CMS and fertile mtDNAs obtained with a range of restriction enzymes. For example, two fragment differences were observed when the *Hin*dIII-generated profiles of the two mitochondrial genomes were compared; the fragments marked "c" and "5.6 kb" in fertile *L. perenne* mtDNA were absent in CMS mtDNA (Fig. 1a). Since the fragment "c" was subsequently demonstrated to be due to plastid DNA contamination (data not shown), there was in fact only a single obvious mtDNA band difference between the two restriction fragment profiles.

The additional 5.6-kb *Hin*dIII fragment that appeared to uniquely identify the fertile *L. perenne* cytoplasm was gel-purified and cloned into the plasmid vector pUC19 to give the derivative pLMF56. Preliminary analyses indicated that pUC19 did not hybridise to *L. perenne* mtDNA (data not shown), thus in all subsequent hybridisations total constructs, i.e. vector plus inserts, were used as probes. pLMF56 was used as a hybridisation probe in a Southern blot analysis of *Hin*dIII-digested mtDNAs from fertile and CMS plants. Figure 2a shows that despite the absence of the 5.6-kb *Hin*dIII fragment in the CMS line (Fig. 1a, lane 2), pLMF56 hybridised to 6.0-kb and 7.4-kb fragments in CMS mtDNA, demonstrating that sequences homologous to the probe were present in the CMS

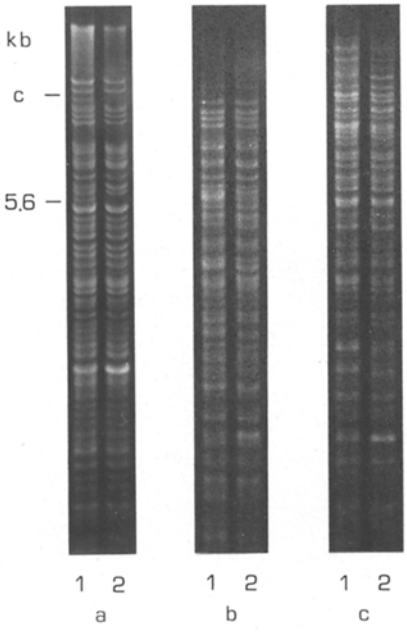

Fig. 1a–c. MtDNA restriction fragment profiles of fertile and CMS *L. perenne*. MtDNA samples were digested **a** *Hin*dIII, **b** *Sac*I and **c** *Pvu*II. *Lane 1* fertile *L. perenne* mtDNA, *lane 2* CMS *L. perenne* mtDNA

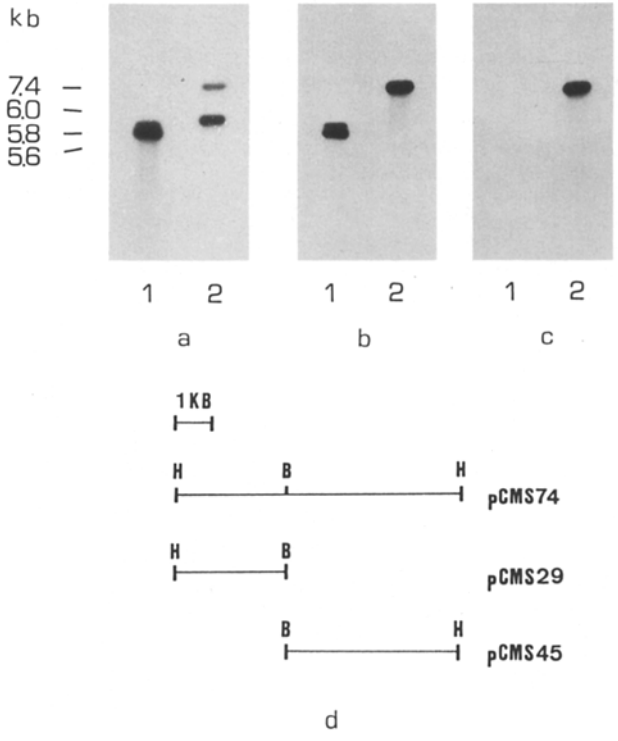

Fig. 2a–d. Southern blot analyses of mtDNAs from fertile and CMS *L. perenne*. Southern autoradiograph of *Hin*dIII-digested mtDNAs probed with **a** pLMF56, **b** pCMS29 and **c** pCMS45. *Lane 1* fertile *L. perenne* mtDNA, *lane 2* CMS *L. perenne* mtDNA. **d** Restriction maps showing the relationship between the probe inserts in pCMS29 and pCMS45 and the insert in pCMS74 from which they were derived. *B*, *Bam*HI; *H*, *Hin*dIII

mitochondrial genome but on different fragments. Furthermore, in addition to detecting the expected 5.6-kb fragment, pLMF56 hybridised to a second fragment of 5.8 kb in mtDNA from fertile *L. perenne*. The complex structural relationships between the various *Hin*dIII fragments which hybridised to pLMF56 in both fertile and CMS mtDNA were analysed in detail and will be published elsewhere (manuscript in preparation). In this report, however, we have confined our analysis to the structure of the 7.4-kb *Hin*dIII fragment detected by pLMF56 in CMS mtDNA and the use of a subclone derived from this fragment as a diagnostic probe for the male-sterile cytoplasm. Clones containing the 7.4-kb *Hin*dIII CMS mtDNA fragment (designated pCMS74) were isolated from shotgun libraries (see Materials and methods) using the gel-purified insert of pLMF56 as the probe.

The digestion of pCMS74 with the restriction enzyme *Bam*HI generated fragments of 2.9 kb and 4.5 kb (Fig. 2d). Each of these fragments was subcloned into pUC19 to give pCMS29 and pCMS45, respectively, and each was then used to probe *Hin*dIII digested purified mtDNAs from the CMS and fertile lines of *L. perenne*. When the probe corresponded to pCMS29, the hybridisation patterns obtained for fertile mtDNA were similar to those obtained using pLMF56 (Fig. 2b, lane 1). However, pCMS29 failed to detect the 6.0-kb *Hin*dIII fragment detected by pLMF56 in CMS mtDNA, hybridising exclusively to the 7.4-kb fragment from which it was derived (Fig. 2b, lane 2). When the filter was reprobed with pCMS45, the same 7.4-kb *Hin*dIII fragment was detected in CMS mtDNA, but no hybridization signal was observed in the lanes containing mtDNA from fertile *L. perenne* (Fig. 2c). This identified pCMS45 as a CMS-specific diagnostic probe that could be used to screen young vegetative plants and thus differentiate between those containing CMS and those with fertile *L. perenne* cytoplasms.

Analysis of progeny using pCMS45

pCMS45 was used to identify the CMS cytoplasm in the progeny of two sets of reciprocal crosses between the maintainer lines Pr146/43 and SB21/46 and the restored-CMS lines 7B273/17 and 6B999/31 (Table 3). Progeny (e.g. A1 and A5) which exhibited full fertility were produced when the maintainer lines were used as female parents (Table 3). When the restored-CMS lines were used as female parents, progeny which segregated for fertility/sterility were produced (e.g. A2 and A6). In addition, the parental line Pr146/43 and 7B273/17 were selfed to give progeny A3 and A4, respectively. Finally, the CMS line 6B1016/19 was crossed as a female parent to the maintainer line T119/46 to produce both sterile (A7 and A8) and fertile (A9 and A10) progeny. Table 3 also lists the predicted cytoplasmic type of the progeny assuming maternal cytoplasmic transmission during all sexual crosses.

Figure 3 shows a Southern blot of *Hin*dIII-digested samples of total cellular DNA enriched for mtDNA probed with pCMS45. The samples include progeny A1–A10 in addition to controls consisting of fertile *L. perenne* and *Festuca pratensis*, the progenitor species from which the *L. perenne* CMS line was originally derived (Connolly and Wright-Turner 1984). Progeny A2, A4 and A6, predicted from genetic analysis to have

Table 3. Details of progeny screened with pCMS45[a]

Parental lines			Progeny		
Female		Male	Name	Phenotype	Predicted cytoplasm
Pr146/43 [F] r r	×	7B273/17 [S] R r	A1	Fertile	F
7B273/17 [S] R r	×	Pr146/43 [F] r r	A2	Segregating	S
Pr146/43 [F] r r	×	Pr146/43 [F] r r	A3	Fertile	F
7B273/17 [S] R r	×	7B273/17 [S] R r	A4	Segregating	S
SB21/46 [F] r r	×	6B999/31 [S] r r	A5	Fertile	F
6B999/31 [S] R r	×	SB21/46 [F] r r	A6	Segregating	S
6B1016/19 [S] r r	×	T119/46 [F] r r	A7, A8	Sterile	S
6B1016/19 [S] r r	×	T119/46 [F] r r	A9, A10	Fertile	S

[a] [S], [F], Sterile and fertile cytoplasms, respectively; R, r, dominant and recessive alleles, respectively, of a nuclear fertility restorer gene controlling male fertility; S, F, fertile and sterile phenotypes, respectively

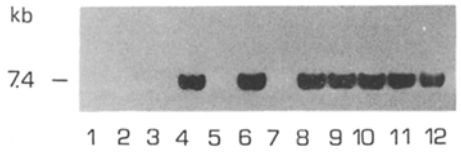

Fig. 3. Southern blot analysis of total cellular DNAs probed with pCMS45. *Lane 1 F. pratensis, lane 2* fertile *L. perenne, lanes 3–12* progeny A1–A10, respectively

inherited the CMS cytoplasm (Table 3), all exhibited a single band of 7.4 kb when hybridised with pCMS45 (Fig. 3, lanes 4, 6, 8), whereas no hybridisation to the pCMS45 probe was evident in progeny A1, A3 and A5, predicted to contain fertile cytoplasm (Fig. 3, lanes 3, 5, 7). Likewise, the fertile control samples from *F. pratensis* and *L. perenne* did not exhibit any hybridisation to pCMS45 (Fig. 3, lanes 1, 2). Furthermore, progeny A7, A8, A9 and A10, all of which were predicted to contain the CMS cytoplasm (Table 3), exhibited the diagnostic 7.4-kb CMS band even though A9 and A10 exhibited a fertile revertant phenotype.

Discussion

The results obtained from our analysis of reciprocal crosses between non-restoring maintainer lines and restored CMS lines together with data indicating stable maternal transmission of male sterility over three sucessive generations confirmed the cytoplasmic mode of inheritance of the character. This prompted the identification and cloning of a CMS mtDNA fragment that when used as a hybridisation probe would be capable of unambiguously distinguishing between the fertile and sterile cytoplasms of *L. perenne*. Because the CMS *L. perenne* line described by Connolly and Wright-Turner (1984) resulted from an intergeneric cross between *Festuca pratensis* and *L. perenne* (followed by repeated backcrossing to *L. perenne*), we considered it likely that any differences between the mtDNA of fertile *L. perenne* lines and the derived CMS line would segregate with the CMS phenotype and might be exploited to generate a CMS-specific probe.

Our initial search for differences between fertile and sterile lines was therefore based on a comparison of restriction enzyme profiles of the respective mtDNAs. Remarkably few differences were observed when fertile and CMS mtDNAs were compared in this way. A single fragment of 5.6 kb represented the major visible difference between the *Hin*dIII digests of mtDNA from fertile and sterile lines. This suggests considerably less rearrangement of the mitochondrial genome than has been reported in most other species where mtDNA from fertile and sterile cytoplasms have been characterised. The restriction enzyme profiles of mtDNA from CMS lines of maize (Borck and Walbot 1982), sorghum (Lee et al. 1989), sugarbeet (Hallden et al. 1990); Duchenne et al. 1989) and radish (Makaroff and Palmer 1988) are markedly different from those of fertile lines. This may indicate that extensive structural rearrangement of the mitochondrial genome has occurred in the CMS derivatives or may reflect the fact that the fertile lines used in the comparisons were not the same as the original fertile progenitor lines. The conservative mtDNA rearrangement in the CMS *L. perenne* line reported here is reminiscent of the kind of minor alterations in mtDNA structure observed between progenitor CMS cytoplasms and fertile revertant cytoplasms such as those described in CMS-T maize by Umbeck and Gengenbach (1983) and in *Brassica* (Bonhomme et al. 1991).

Although originally identified on the basis of its absence in *Hin*dIII digests of CMS mtDNA, the cloned 5.6-kb *Hin*dIII fragment (designated pLMF56) detected homologous sequences in both types of mtDNA when used as a hybridisation probe. Surprisingly, molecular cloning and analysis of the mtDNA fragments detected by pLMF56 in the CMS line led to the identification of a 4.5-kb *Hin*dIII-*Bam*HI fragment, cloned as pCMS45, which did not detect homologous sequences in the mtDNA of the fertile line. Mitochondrial DNA sequences which are apparently unique to CMS lines have also been reported in other species. The mitochondrial genomes of an isonuclear fertile and CMS derivative of sunflower are almost identical except for an 11-kb inversion and 5-kb insertion in the CMS line (Sicullela and Palmer 1988; Laver et al. 1991). Southern hybridisation experiments using mtDNA probes derived from the CMS-specific insertion indicated that these sequences were absent in the mtDNA of fertile lines (Kohler et al. 1991). The source of these apparently unique CMS-specific sequences has not yet been identified unequivocally. Small et al. (1989) have suggested that the appearance of hitherto unobserved sequences in plant mitochondrial genomes may be a consequence of recombination events that lead to the integration of low copy number mtDNA sequences, present in the progenitor cytoplasm, into the main mitochondrial genome.

The isolation of pCMS45 provided a simple diagnostic test for the positive identification of young non-flowering plants carrying the CMS cytoplasm amongst progeny of reciprocal test crosses involving fertility-restoring and maintainer lines of *L. perenne*. It also permitted confirmation at the molecular level of the maternal inheritance of the CMS trait suggested by the genetic data. This was especially relevant to the CMS *L. perenne* system because it appears to have originated following a rare paternal cytoplasmic inheritance event (manuscript in preparation).

Progeny A9 and A10, derived from a cross between a CMS line as the female parent and a maintainer line as the male parent, were expected to exhibit sterility, but were in fact fertile, suggesting that either paternal cytoplasmic inheritance or reversion to fertility had occurred. However, since A9 and A10 appeared to contain the CMS cytoplasm, based on the presence of the diagnostic CMS mtDNA fragment detected by pCMS45, paternal cytoplasmic inheritance could be ruled out. Thus, it is clear that this particular fertility reversion event is unaffected by the presence of sequences homologous to pCMS45. This suggests a number of possible explanations. The CMS-specific sequences may not, for example, play a causative role in the CMS phenotype but merely act as a genetic marker for the sterile cytoplasm. If, on the other hand, the CMS-specific sequences are involved directly in the CMS phenotype, then reversion to fertility might be the result of a mutation that supresses their effect. The occurrence of just such a mutation has been correlated with reversion to fertility in a CMS-T line in maize (Wise et al. 1987).

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