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Alloplasmic male sterility in AD allotetraploid *Gossypium hirsutum* upon replacement of its resident A cytoplasm with that of D species *G. harknessii*

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Summary. Alloplasmic male sterile (cms) and restoration-of-fertility (*Rf*) lines of the AD allotetraploid *Gossypium hirsutum* were earlier derived from the presumed introgression of the cytoplasm of the D species *G. harknessii*. To confirm that this happened and address its significance, cytoplasms of the maternal progenitor, backcross intermediates, derived breeding lines, related A, D, and F species, and a synthetic AD tetraploid were examined by agarose and polyacrylamide gel electrophoresis of 140 restriction enzyme fragments of chloroplast DNA. Length mutations of 10–50 nucleotides predominate over site loss/gain mutations. Chloroplast DNA is maternally inherited and that of *G. harknessii* has been maintained in the cms lines for at least 13 successive generations without detectable alteration. Chloroplast DNA divergence is consistent with current nuclear genome classification and shows that the A progenitor was the maternal parent of the AD tetraploids. As predicted from incompatability models of cms, the degree of male sterility in alloplasmic *Gossypium* tetraploids is correlated with the extent of evolutionary divergence of their cytoplasms. It is suggested that the A genome in the AD tetraploids dominates those nuclear-cytoplasm interactions reflected by male fertility.

Key words: *Gossypium* chloroplast DNA – Maternal inheritance – Cytoplasm classification – Allotetraploid maternal ancestor – Alloplasmic male sterility

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Introduction

Cytoplasmic male sterility (cms) is a cytoplasmically inherited trait characterized by failure to produce viable pollen. It may occur spontaneously or in alloplasmic plants resulting from intergeneric, interspecific, or intraspecific cross-pollination (Edwardson 1970). The alloplasmic cms phenotypes are often pleiotropic and restoration of male fertility in such plants is ascribed to the maintenance of a cytoplasm-homologous nuclear gene in the foreign nuclear genome (Edwardson 1970; Hanson and Conde 1985). It has been postulated that cms, regardless of origin, is due to a nuclear-cytoplasmic incompatability and that different mechanisms are likely to exist in different cms systems (Edwardson 1970; Flavell 1974; Hanson and Conde 1985). Those best described arise from mutations in mitochondrial DNAs (Hanson and Conde 1985; Pring and Lonsdale 1985; Schardl et al. 1985; Young and Hanson 1987; Dewey et al. 1986; Smith et al. 1987).

Alloplasmic male sterility in cotton derives from an interspecific cross between the D diploid, *G. harknessii*, as maternal parent with the AD allotetraploid, *G. hirsutum* (Meyer 1975). After thousands of pollinations onto the sterile triploid hybrid, one plant was recovered from which cms and restorer lines were developed by recurrent backcrossing with *G. hirsutum* as pollen parent (Fig. 1). The cms and restorer lines should both be essentially *G. harknessii* cytoplasm in a *G. hirsutum* nuclear background with the *Rf* gene in the restor line deriving from *G. harknessii*. For ease in breeding, the *Rf* gene is maintained in the otherwise cms cytoplasm.

Flower morphology is essentially normal in *G. harknessii*-derived cms except that flowers and anthers are reduced in size and anthers lack pollen. There is premeiotic collapse of sporangeous tissue, disor-

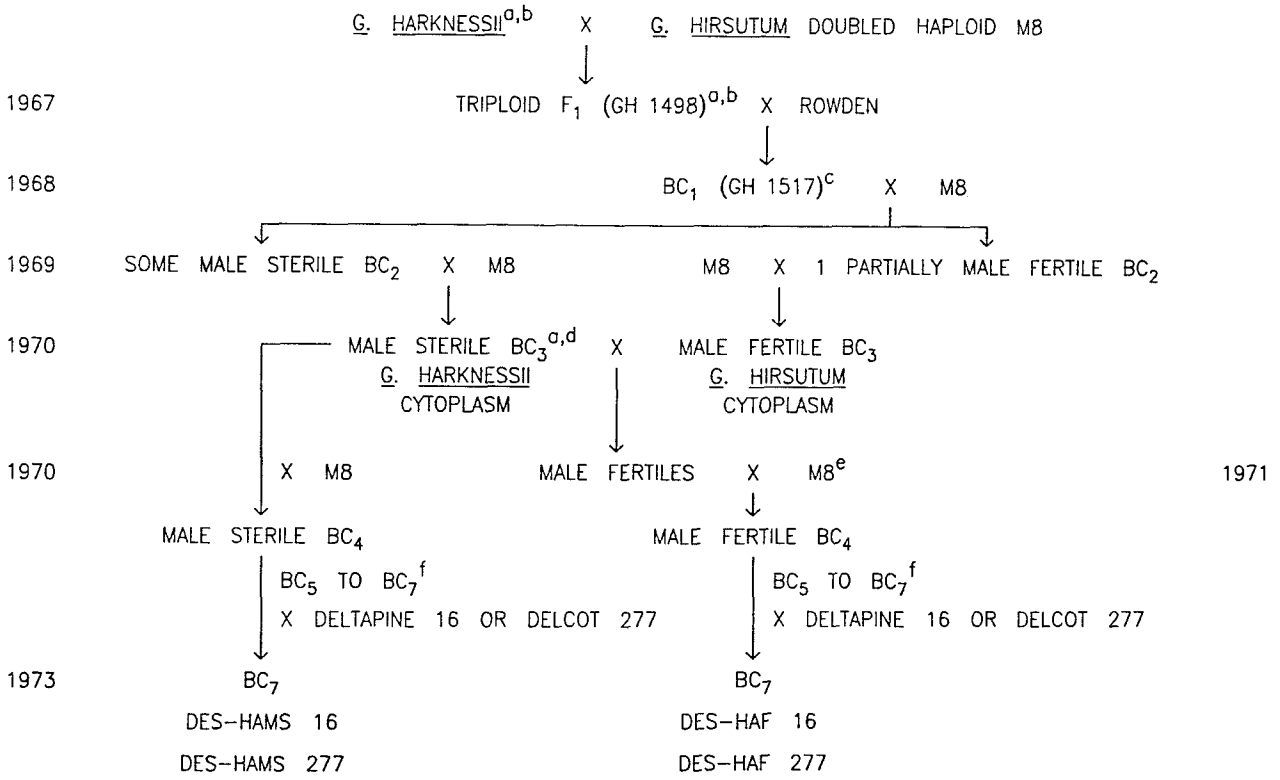

Fig. 1. Pedigree of cms and restorer lines (Meyer 1973 a, b, 1974, 1975, unpublished results). Rowden, Deltapine 16 and Delcot 277 are *G. hirsutum* cultivars; [a] plant maintained by J. McD. Stewart (Table 1); [b] cutting maintained at the University of Georgia; [c] the triploid F_1 resisted chromosome doubling to a fertile hexaploid (Meyer 1974). Two BC_1 seed were produced after thousands of crosses onto the triploid (Meyer 1973 b); [d] two completely male sterile BC_3 progeny were used; [e] a BC_4 generation of DES-HAF were obtained from the winter nursery at Iguala, Mexico. With this exception, all backcrosses starting with BC_2 were presumably conducted in the field at Stoneville, MS; [f] the number of backcrosses to cultivars and, hence, the total number of backcrosses are not consistent with the time of release

ganization of tapetal cells, and rare pollen mother cells degenerate during early prophase of meiosis (Murthi and Weaver 1974). Sporophytic restoration of male fertility appears to be contingent on the single dominant *Rf* gene (Meyer 1973 b; Weaver and Weaver 1977) present in the *Rf* lines and presumably the *G. harknessii* progenitor. Restoration in *Rfrf* hybrids is somewhat variable in *G. hirsutum* but complete in backrounds of another allotetraploid *G. baradense* (Meyer 1975; Sheetz and Weaver 1980). A dominant fertility modifying gene *E* from *G. barbadense*, termed the Pima enhancer factor, confers in *G. hirsutum* restorer lines complete fertility in F_1 hybrids that are *RfrfEe* (Sheetz and Weaver 1980).

In contrast, several AD, A, or A-related cytoplasms are generally male fertile in these backgrounds (Meyer 1973 a; Meredith et al. 1979; Mahill 1982). The principal objectives of the present work were to classify the cytoplasms of these *Gossypium* species, determine if *G. harknessii* cytoplasm was in fact inherited in the cms lines, and determine what type of cytoplasm it replaced. Restriction enzyme analysis of chloroplast DNAs distinguishes *Gossypium* cytoplasms and demonstrates that the D-type *G. harknessii* cytoplasm was inherited in the cms and restorer lines without detectable alteration in plastid DNA. It replaced a resident A-type cytoplasm, showing that the degree of divergence in cytoplasms is correlated with the degree of male sterility in AD tetraploids. It also suggests that the nuclear A subgenome in the tetraploids controls those nuclear-cytoplasmic interactions responsible for male fertility.

Materials and methods

Species, cultivars, and breeding lines

Table 1 lists the plants examined. Preparation of chloroplast DNA used single plants of the interspecific hybrid, the BC_3 derivative, most diploids, 2 plants of *G. arboreum* AKH and at least 4 plants with tetraploid background.

Isolation of chloroplast DNA

Working solutions were made with stocks of 1 *M* TRIS-HCl and 0.4 *M* EDTA-NaOH, both at pH 8.0 at room temperature. The indicated pH of solutions is that at 4 °C. All components were made 0.1% diethylpyrocarbonate and autoclaved.

Table 1. Description of plants used as sources of chloroplast DNA

Figure code	Nuclear background		Cytoplasm	Source and accession[a]	Athens code
	Species, cultivar, or line	Genome			
HERB	*G. herbaceum* × *G. harknessii*	$A_1 \times D_{2-2}$	*G. herbaceum*	JMS; CB2487 × A-15	36
	G. herbaceum var *africanum*	A_1	*G. herbaceum*	JMS; VM seed	39
ARB	*G. arboreum*	A_2	*G. arboreum*	AEP; AKH-235 seed	8
	G. arboreum var *sanguineum*	A_2	*G. arboreum*	JBW; 1981 seed	$12S_2$
LONG	*G. longicalyx*	F_1	*G. longicalyx*	AEP; G seed	28
THUR	*G. thurberi*	D_1	*G. thurberi*	AEP; CB3782 seed	6
RAIM	*G. raimondii*	D_5	*G. raimondii*	AEP; 9 seed	1
HARK	*G. harknessii*	D_{2-2}	*G. harknessii*	JMS; VM 1983 seed	32
	G. harknessii	D_{2-2}	*G. harknessii*	JMS; A-15	91
HIR	*G. hirsutum* Coker 315	$(AD)_1$	*G. hirsutum*	JBW; 1983 seed	
	G. hirsutum Dixie King *ne*	$(AD)_1$	*G. hirsutum*	JBW; 1982 seed	
	G. hirsutum G8160	$(AD)_1$	*G. hirsutum*	JBW; 1981 seed	
BARB	*G. barbadense* Pima 79-106, *E*	$(AD)_2$	*G. barbadense*	JBW, BC_{3-4} 1983 seed	
	G. barbadense Egyptian Pima	$(AD)_2$	*G. barbadense*	DWA; B-110 seed	$56S_1$
HAS-BC4	*G. hirsutum* BC_3 × G8160, cms	$(AD)_1$	*G. harknessii*	JMS; 1983 seed	41
HAS-278	*G. hirsutum* 5-278, cms	$(AD)_1$	*G. harknessii*	JBW; 1978 seed	
HAS-G81	*G. hirsutum* G8169, cms	$(AD)_1$	*G. harknessii*	JBW; BC_6 1982 seed	
HAS-825	*G. hirsutum* Stoneville 825, cms	$(AD)_1$	*G. harknessii*	JBW; BC_4 1982 seed	
HAS-DK	*G. hirsutum* Dixie King *ne*, cms	$(AD)_1$	*G. harknessii*	JBW; BC_4 1982 seed	
HAF-DK	*G. hirsutum* Dixie King *ne*, *Rf*	$(AD)_1$	*G. harknessii*	JBW; 1983 seed	
HAF-277	*G. hirsutum* DES-HAF 277, *Rf*	$(AD)_1$	*G. harknessii*	JBW; VM, 37 1978 seed	
HAS-BARB	*G. barbadense* Pima 79-106 *E*, cms	$(AD)_2$	*G. harknessii*	JBW; BC_{3-4} 1982 seed	
HAF-BARB	*G. barbadense* Pima 79-106 *E*, *Rf*	$(AD)_2$	*G. harknessii*	JBW; BC_{3-4} 1982 seed	

[a] JMS, J. McD. Stewart, Department of Agronomy, University of Arkansas, Fayetteville, Arkansas 72701 USA; AEP, A. E. Percival, and DWA, D. W. Altman, USDA/ARS, P. O. Drawer NN, College Station, Texas 77841 USA; JBW, J. B. Weaver, Jr., Department of Agronomy, University of Georgia; VM, V. A. Meyer

Leaves of greenhouse-grown plants (150–250 g) were rinsed in cold 10% bleach, 1% ethanol, rinsed extensively in water, and cut into small pieces and were stored at −80 °C after freezing in liquid nitrogen. Tissue was homogenized 15–30 sec in a blender at maximum speed with 1.5 l HB (0.35 *M* sorbitol, 50 m*M* TRIS-HCl, 10 m*M* EDTA-NaOH, 0.1% bovine serum albumin, 5 m*M* 2-mercaptoethanol, pH 8.3), filtered through three layers of cheesecloth and one layer of miracloth, and centrifuged at 4,000 rpm (3,000 × *g*). Pellets were suspended in a total of 25 ml WB (identical to HB buffer except containing 10 m*M* TRIS-HCl and 50 m*M* EDTA-NaOH, pH 8.5), and 6–7 ml aliquots were layered over each of four tubes containing 12 ml 0.9 *M* sucrose over 18 ml 1.75 *M* sucrose, both in 50 m*M* TRIS-HCl, 20 m*M* EDTA-NaOH, 5 m*M* 2-mercaptoethanol, pH 8.2, and centrifuged at 13,000 rpm (27,000 × *g*) for 30 min. The chloroplasts were collected from the sucrose/sucrose interface, diluted to 200 ml with WB and again concentrated, fractionated on sucrose, and concentrated as before. All steps were at 4 °C.

The final chloroplast pellet was suspended in 36 ml 50 m*M* TRIS-HCl, 20 m*M* EDTA-NaOH, 600 µg/ml ethidium bromide, pH 8.2. The chloroplasts were lysed at room temperature by the addition of 12 ml 20% Sarkosyl. CsCl was added to 1.58 g/cm^3 (about 52 g) and the solution was centrifuged in two tubes for 40 h at 40,000 rpm at 20 °C in a Beckman 70Ti rotor. The single DNA band was recovered by usual methods. Yields averaged 0.3 and 2 µg/g fresh weight for diploids and tetraploids respectively, about a 3%–15% recovery (Galau et al. 1988).

Restriction fragment analysis

Aliquots of 5 µg DNA were incubated at 37 °C in 30 µl containing recommended buffer, restriction enzyme (Bethesda Research Labs.), and 6 m*M* spermidine-HCl (Bouche 1981), and frozen when completely digested. Criteria were that the fragment pattern was not changed with increased enzyme levels and was the same for all preparations from the same species, and that lambda DNA was completely digested when included with the *Gossypium* DNAs. For electrophoresis, 0.3–0.5 µg aliquots were adjusted to 0.05% bromophenol blue, 0.05% xylene cyanole FF, 5% Ficoll, 40 m*M* EDTA-NaOH, 0.5 × digestion buffer, and were heated 5 min at 65 °C. Electrophoresis was in 0.8%–1.0% agarose submarine gels (3 mm × 28 cm) for 6 h at 5.4 V/cm or in 5% T (2.6% C) polyacrylamide vertical gels (1.6 mm × 25 cm) for 10 h at 4 V/cm, both run in TBE (90 m*M* TRIS base, 90 m*M* boric acid, 2.5 m*M* Na_2EDTA, pH 8.3), 0.5 µg/ml ethidium bromide.

Results

Classification of species cytoplasms with chloroplast DNA

The Chloroplast DNAs of *G. thurberi*, *G. raimondii*, *G. harknessii*, *G. barbadense*, *G. hirsutum*, and *G. herbaceum* × *G. harknessii* were completely cut with BamHI,

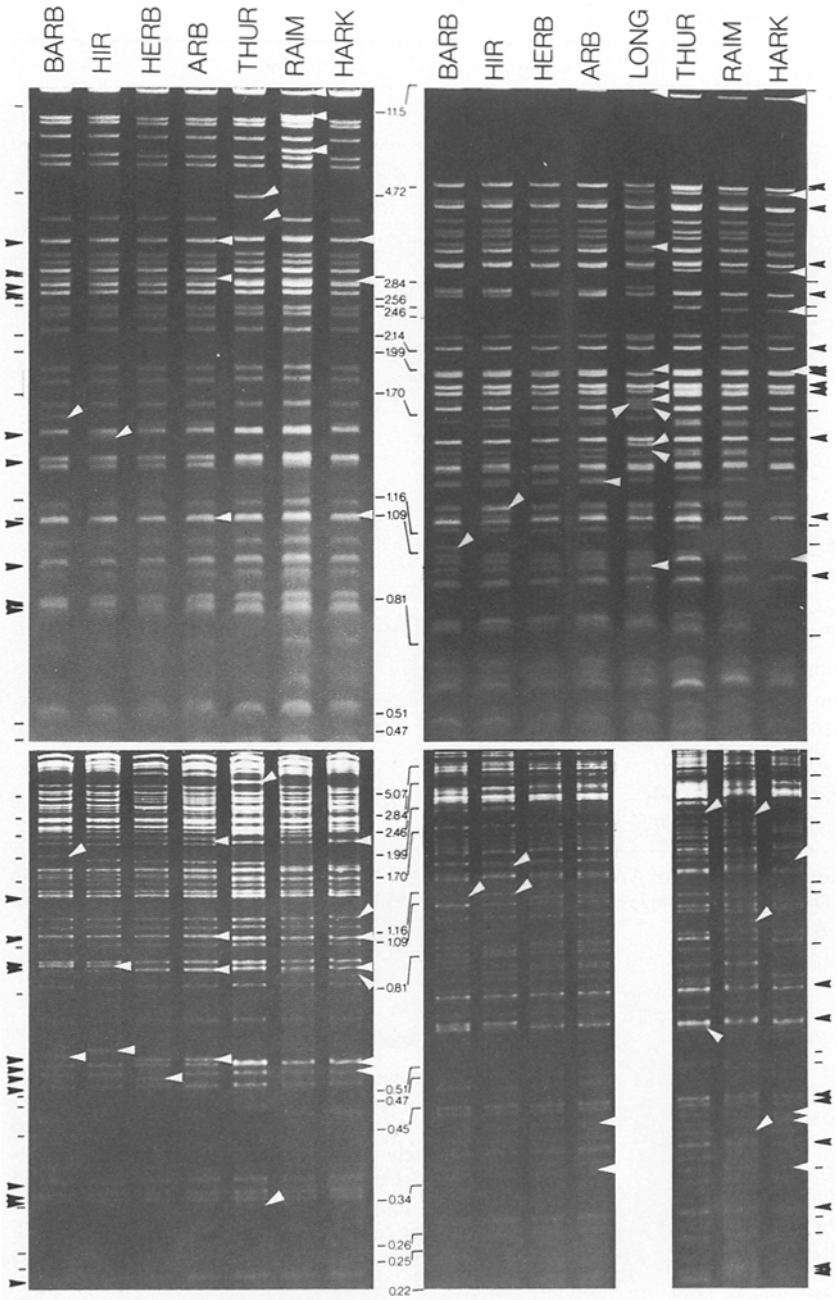

Fig. 2. Fragment patterns of *Gossypium* chloroplast DNAs. Chloroplast DNAs were digested with BamHI (left) and EcoRI (right) and fractionated on agarose (top) and polyacrylamide (bottom) gels. Genome abbreviations are described in Table 1. The IVR fragments in exterior lanes are indicated by filled *arrows*. Marker lanes (not shown) contained Pst I-digested lambda DNA; their positions are indicated as are their sizes in kb. Inclined *arrows* indicate species-specific differences and horizontal *arrows* other mutations listed in Table 2. The bottom of each panel has been underexposed during printing to enhance the smaller fragments

EcoRI, and HindIII and electrophoresed in both agarose and polyacrylamide gels, in order to enhance the ability to discriminate species-specific differences in DNA fragments. Analysis of *G. arboreum* (BamH and EcoRI on both gel systems) and *G. longicalyx* (EcoRI on agarose only) was less extensive. Examples of these gels are shown in Fig. 2. The migration rate and stoichiometry of each fragment was compared for all species in multiple exposures of these and other gels that had different orders of load. Both agarose and polyacrylamide gels have reasonable resolution of 1.0–1.5 kb fragments and virtually all fragments of this size could be identified in each, even though there are differences in their relative migration rates.

The DNAs are clearly those of chloroplasts. Table 2 summarizes their genome size, assigning those fragments with twice the normal intensity in both gel systems (where applicable) to the inverted repeat (IVR) of the genome (shown in Fig. 2). The fragment number and genome size of about 148 kb is similar for all species with the same enzyme, and the genome size is essentially independent of the enzyme, indicating at least a fair reliability

Table 2. *Gossypium* chloroplast DNA fragments and some of their mutations

Enzyme	Genome and species[a]							
	(AD)		A		F	D		
	BARB	HIR	HERB	ARB	LONG	THUR	RAIM	HARK
BamHI								
No. non-IVR fragments	27	27	25	25		28	28	27
No. IVR fragments	17	17	17	17		17	17	17
IVR, kb	23	23	23	23		23	23	23
all, kb	148	148	147	147		150	152	147
Mutations, kb								
IVR	3.47	3.47	3.47	3.47		3.52	3.52	3.52
	1.13	1.13	1.13	1.13		1.12	1.12	1.12
	0.88	0.88	0.87	0.87		0.875	0.875	0.875
	0.60	0.62	0.58	0.58		0.57	0.57	0.57
	0.55	0.55	0.53	0.55		0.55	0.55	0.55
Non-IVR	16	16	16	16		16	9.8+6.8	16
	2.82	2.82	2.82	2.82		2.77	2.77	2.77
	–	–	–	–		2.23	2.23	2.23
EcoRI								
No. non-IVR fragments[b]	51	51	52	52		52	49	47
No. IVR fragments[b]	17	17	17	17		17	17	17
IVR, kb[b]	30	30	30	30		30	30	30
all, kb[b]	143	145	146	144		146	143	145
Mutations, kb								
IVR	1.98	1.98	1.98	1.98	1.90	1.98	1.98	1.98
Non-IVR	10.8	10.8	10.8	10.8	10.8	10.2	10.2	10.2
	–	–	–	–	4.75	4.75	4.75	4.75
	3.45	3.45	3.45	3.45	3.45	–	–	–
	–	–	–	–	–	2.56	2.56	2.56
	1.74	1.74	1.74	1.74	1.74	–	–	–
	1.33	1.33	1.33	1.33	–	–	–	–
	1.035	1.035	1.035	1.035	1.035	1.055	1.055	1.055
	–	–	–	–		0.42	0.42	0.42
	0.415	0.415	0.415	0.415		–	–	–
	–	–	–	–		0.41	0.41	0.41
	0.345	0.345	0.345	0.345		0.34	0.34	0.34
HindIII								
No. non-IVR fragments	23	23	22			22	23	22
No. IVR fragments	6	6	6			6	6	6
IVR, kb	24	24	24			24	24	24
all, kb	151	151	151			153	154	153
Mutations, kb								
IVR	1.38	1.38	1.38			1.37	1.37	1.37
Non-IVR	1.27	1.27	1.27			1.29	1.29	1.29

[a] Abbreviations are listed in Table 1

[b] Only approximate due to large number of fragments

in the fragment assignments and their lengths. The IVR size estimates of about 25 kb are somewhat dependent on enzyme, as expected. Both sizes are typical of higher plant chloroplast DNAs (Palmer 1985).

Inspection of Fig. 2 shows that the chloroplast DNA of each species can be distinguished. Some of the species-specific fragment differences are shown by inclined arrows in Fig. 2. Although only single accessions of three species were examined, intraspecific variation is probably minor, since no differences were found in less extensive analysis among three cultivars of *G. hirsutum*, two Pima varieties of *G. barbadense*, and two accessions each of *G. harknessii*, *G. arboreum* and *G. herbaceum* (listed in Table 1; data not shown). From comparison to *G. herbaceum* and *G. harknessii* (data below and not shown), the DNA of *G. herbaceum* × *G. harknessii* is that of the maternal parent *G. herbaceum*.

The differences in species chloroplast DNA fragments are consistent with their nuclear genome designations. Only 1 of 111 EcoRI and BamHI fragments are

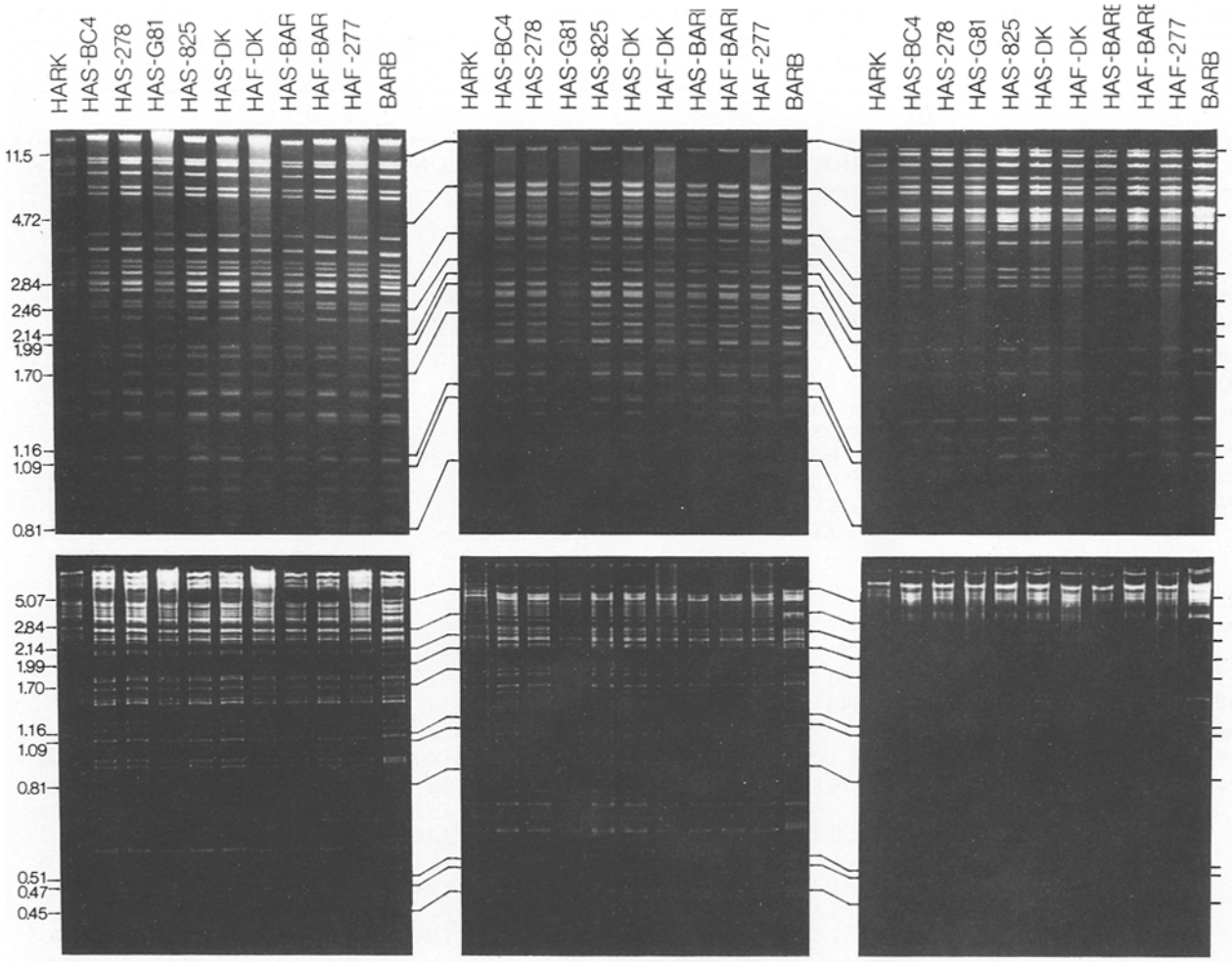

Fig. 3. Chloroplast DNAs of *G. harknessii* and derivatives of *G. harknessii* × *G. hirsutum*. Chloroplast DNA was digested with BamHI (left), EcoRI (middle), and HindIII (right) and fractionated on agarose (top) and polyacrylamide (bottom) gels. Other details are as in Fig. 2

different between *G. arboreum* and *G. herbaceum*, about 15% of fragments are different between the two AD species, 20% between the three D species, 20% between A and AD species, 40% between D and A or AD species, and about 10% between A species and the single F species (Fig. 2; data not shown). An evolutionary tree cannot be made with these enzymes, since the fragment complexity precludes assignment of most of the fragment differences to specific site loss/gain or length mutations consisting of insertion/deletion events or conformational nucleotide substitutions seen in polyacrylamide gels (Singh et al. 1987). Most of the observed fragment differences with BamHI and HindIII are probably due to length mutations (horizontal arrows in Fig. 2; Table 2); at least 10 of 13 obvious events that are detected with these enzymes are very likely fragment length differences of 5–80 nucleotides.

The mutations listed in Table 2 classify the cytoplasms in the same way as overall fragment-difference coefficients. The A cytoplasms are distinguished from the D cytoplasms by all of the 17 phylogenically informative mutations. Since the AD cytoplasms nearly always share the same sized fragments with the A cytoplasms and not the D cytoplasms, it is clear that the AD tetraploids contain an A-like cytoplasm and, consequently, the maternal ancestor of the tetraploids was the A species progenitor. Although data are more limited for the F species, it appears to be more similar to the A rather than D species.

Maternal inheritance of G. harknessii chloroplast DNA in the cms lines without detectable alteration

Of primary interest was to confirm that *G. harknessii* cytoplasm was inherited in cms and restorer lines and to see if its chloroplast DNA was altered upon transfer to *G. hirsutum* and *G. barbadense* nuclear backgrounds. Nine derivatives of the *G. harknessii* plant used by Meyer

(Table 1; Fig. 1) were examined. These included progeny of the *G. harknessii* individual, the BC_3 male sterile selection × G8160 *gl*, a selection from the DES-HAF 277 release, and other recent cms, *Rf*, and *E* breeding lines in *G. hirsutum* and *G. barbadense* backgrounds (Table 1). The fragment analysis shows that all contain the same chloroplast DNA (Fig. 3) and it is the same as that present in another *G. harknessii* accession (data not shown). We conclude that *G. harknessii* chloroplast DNA was maternally inherited without alteration in the backcrossing development of the alloplasmic male-sterile lines. The results also demonstrate that, as expected, the inclusion of nuclear genes *Rf* and *E* that reversibly modify male fertility do not detectably alter the structure of the chloroplast genome.

Discussion

Restriction fragment polymorphisms in *Gossypium* chloroplast DNA identify species and nuclear genome group cytoplasms and indicate that the tetraploids contain A cytoplasms. Meyer's male-sterile and restorer introgressions all contain *G. harknessii* cytoplasm from the maternal progenitor, as has been presumed, resulting in male sterility upon replacement of the resident A cytoplasm of the AD tetraploid by a typical D cytoplasm.

Gossypium harknessii chloroplast DNA in the cms and restorer lines

Results from both *G. harknessii* × *G. hirsutum* derivatives and the *G. herbaceum* × *G. harknessii* hybrid demonstrate that *Gossypium* chloroplast DNA is maternally inherited. This has been predicted from earlier experience with presumed plastid mutations (Krishnaswami 1968) and interspecific crosses (Meyer 1973b, 1974). With the *G. harknessii*-derived cms lines, for instance, fertile progeny of cms × maintainer are at most exceedingly rare, indicating that there must be a very low frequency of paternal inheritance that should restore fertility. Maternal inheritance of chloroplast DNA in *Gossypium* has also been deduced from the inheritance of the large subunit of ribulose bisphosphate carboxylase (Chen and Wildman 1981), a polypeptide encoded in higher plants by chloroplasts. All these results are consistent with the reported absence of plastids in *G. hirsutum* generative cells (Corriveau and Coleman 1988).

Changes in chloroplast DNA have been inferred to have occurred during two interspecific cytoplasm introgressions in *Nicotiana* (Frankel et al. 1979). No change in 136 fragments in *G. harknessii* chloroplast DNA was detected in the eight lines derived from *G. harknessii* × *G. hirsutum*. These represent approximately 45 independent backcross generations, including about 13 successive generations in the development of cms G8160 *ne*. Selected mutations (considering the thousands of pollinations on the F_1) or cross-induced mutations may still have occurred, since only 0.6% of the nucleotides were directly examined. However, insertion/deletion events of more than 50 nucleotides or inversions of more than 500 nucleotides are virtually precluded, as they should have been exposed in fragments produced with at least one of the three enzymes that were used.

Allotetraploid cytoplasms are A-type

The preliminary analysis of differences in chloroplast DNAs agrees with the usual view of species divergence in the genus. The approximately 40 species are divided into genome groups primarily on the basis of chromosome pairing and chiasma frequency in interspecific hybrids, chromosome size, plant morphology, and distribution (Endrizzi et al. 1985). Both of the A genome diploids were examined and their chloroplast DNAs were found to be very similar and much more closely related to those of the AD allotetraploids (2 examined of 5–6 described) than those in the D genome diploids (3 examined of 12 described). Consequently, the A genome progenitor of the two AD allotetraploids was the maternal parent, as has also been suggested by Chen and Wildman (1981) from similarities in the large subunit of ribulose bisphosphate carboxylase. The cytoplasm of the maternal progenitor was probably more closely related to *G. arboreum* than to *G. herbaceum*, since the single detected difference between *G. arboreum* and *G. herbaceum* is a deletion in the BamHI 0.55-kb IVR fragment unique to *G. herbaceum*. It is generally agreed, however, that the nuclear genome of *G. herbaceum* is more closely related to the A genome in the AD species (Endrizzi et al. 1985).

Cytoplasm divergence, cms, and Rf in alloplasmic Gossypium

It is not known which organelle is responsible for cms in *Gossypium*, but chloroplast DNA divergence should be a rough index of cytoplasm phenotype and mitochondrial and nuclear DNA divergence (Frankel et al. 1979; Kung et al. 1982; Bowman et al. 1983; Terachi and Tsunewaki 1986). Co-evolution of cytoplasmic and nuclear components of male fertility is confirmed by the results presented here. Extensively backcrossed progeny containing *G. herbaceum*, *G. arboreum*, *G. longicalyx*, *G. barbadense*, or *G. hirsutum* cytoplasms in either *G. hirsutum* or *G. barbadense* nuclear backgrounds are male fertile (Meyer 1973a; Meredith et al. 1979; Mahill 1982). All these cytoplasms are A or A-like. In contrast, the D cytoplasm from *G. harknessii* results in male sterility.

Apparently the D subgenome in the tetraploids is now not fully compatible with D cytoplasms, while the A subgenome is still compatible with A-like cytoplasms.

This inference further suggests that, with its resident cytoplasm, the A subgenome is primarily responsible for those nuclear-cytoplasmic interactions whose failure in other cytoplasms leads to male sterility. All species examined here, as well as others, have been screened for the presence of nuclear genes that can substitute in *G. harknessii* cytoplasm-containing tetraploids for the *Rf* gene presumed to be derived from *G. harknessii* (Meyer 1975). None were found, even in *G. thurberi, G. raimondii,* and other D species whose cytoplasms are probably more similar to that in *G. harknessii* than between those of the AD, A, and F species that are male fertile in AD backgrounds. If both of these preliminary results are confirmed, they would also suggest that fertility alleles in the D genome function poorly in tetraploids. The synthesis of additional alloplasmic tetraploids (J. McD. Stewart, personal communication), a detailed chloroplast phylogeny (J. F. Wendel, personal communication), and examination of mitochondrial genomes should help further explore the extent and history of the apparent dominance of the A subgenome.

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