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The plant mitochondrial genome: homologous recombination as a mechanism for generating heterogeneity

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The mitochondrial genomes of higher plants are among the largest and most complex organelle genomes described. They are generally multicircular or partly linear; in some species, extrachromosomal plasmids are present. It is proposed that inter- and intramolecular homologous recombination can account for the diversity of the observed genome organizations. The ability of mitochondria to fuse establishes a panmictic mitochondrial DNA population which is in recombinational equilibrium. It is suggested that this suppresses the base mutation rate, and unequal partitioning of the cytoplasm during cell division can lead to the rapid evolution of mitochondrial genome structure. This contrasts with the observed rates of base-sequence and genome evolution in chloroplasts. This difference can be accounted for solely by the inability of chloroplasts to fuse, thereby preventing chloroplast genome panmixis.

Introduction

Most but not all eukaryotes contain mitochondria (Cavalier-Smith 1987). In those that do, the mitochondrion is known to contain its own unique genetic system. In the Metazoa, mitochondrial genomes are remarkably uniform in size and structure, being simple circular molecules of between 15 and 17 kilobases (kb). In other organisms, both unicellular and multicellular, mitochondrial genome size and organization vary considerably. In the unicellular green alga *Chlamydomonas reinhardtii*, the mitochondrial genome is a linear molecule of 15.8 kb (Boer & Gray, this symposium). Linear genomes are also found in other organisms, for example in the protozoan *Tetrahymena thermophila* (Morin & Cech 1986), and the yeast species *Candida rhagii* (Kovac *et al.*, 1984) and *Hansenula mrakii* (Wesolowski & Fukuhara 1981). In the algae, Protozoa, the majority of the fungi and the higher plants, circular mitochondrial genomes predominate. In fungi, genome sizes range from 19 to 176 kb (Clark-Walker *et al.* 1981; Hintz *et al.* 1985); these genome sizes are, however, small compared with the mitochondrial genomes of higher plants, which range from 200 kb to over 2400 kb (Ward *et al.* 1981). The complexity of mitochondrial genome organization appears to increase with increasing size.

In the fungi the mitochondrial genomes can become defective. Such defective genomes are usually amplified plasmid populations or amplified deletion derivatives of the mitochondrial genome; they are generally suppressive towards the parental mitochondrial DNA (mtDNA) and result in an altered growth phenotype. Examples include the petite mutants of yeast, *Saccharomyces cerevisiae* (Dujon 1981), the poky and stopper mutants of *Neurospora* (Manella *et al.* 1979; Bertrand *et al.* 1980), the ragged mutant of *Aspergillus amstelodami* (Lazarus & Kunzel 1981) and the senescent strains of *Podospora* (Wright *et al.* 1982; Bockelmann & Esser

1986). These defective molecules usually arise by a process of recombination between short reiterated sequences (Clark-Walker *et al.* 1981; de Zamaroczy *et al.* 1983).

In some species of fungi and higher plants, the mitochondria contain plasmids that replicate independently of the mitochondrial genome. These are of either DNA or RNA, are circular or linear and have little or no homology with the genome of high molecular mass. The mitochondrial genomes of higher plants show yet another facet of organizational complexity; electron microscopy demonstrates that the DNA isolated is mostly linear, circular species being rare. The linear forms observed by electron microscopy are probably artefacts of the isolation procedure, as restriction mapping studies suggest that the genomes are, with the exception of *Brassica hirta*, multicircular. Alternatively, the mitochondrial genomes can be represented as a single circular molecule, from which all the other smaller circles, excluding the circular plasmids, originate.

In this paper, we consider mitochondrial genome organization in higher plants and how its organization is derived, maintained and inherited.

TABLE 1. CIRCULAR PLASMID DNAS IN MITOCHONDRIA OF HIGHER PLANTS

species	size/kb (bp)[a]	reference
Zea mays		
N, CMS-C,T,S	1.9 (1913)	Kemble & Bedbrook (1980)
	1.4	Dale *et al.* (1981)
CMS-C	1.5	Ludwig *et al.* (1985)
CMS-C	1.4	
Beta vulgaris	1.3 (1308)	Powling (1981)
	1.4 (1440)	Hansen & Marcker (1984)[a]
	1.45	Thomas (1986)[a]
	1.5 (1620)	
CMS 01I13M4	7.3	
Phaseolus vulgaris	1.9	Dale *et al.* (1981)
Vicia faba	1.4 (1476)	Goblet *et al.* (1983)
	1.75 (1704)	Goblet *et al.* (1985)
	1.75 (1695)	Nikiforova & Negruk (1983)
(CMS 350)	1.5	Negruk *et al.* (1985)
		Wahleithner & Wolstenholme (1987)[a]
Helianthus annua	1.45	Leroy *et al.* (1985)
Sorghum bicolor	2.3	Chase & Pring (1985)
	1.7	
	1.36	

[a] Size given in base pairs (bp) where plasmid sequence has been reported.

Circular plasmids associated with plant mitochondria

Small circular DNA species, sometimes referred to as plasmids or minicircular DNAs (Sederoff 1984; Pring & Lonsdale 1985) are by far the most abundant extrachromosomal elements associated with plant mitochondria but as yet have been identified in only six species (table 1). They are best visualized by running unrestricted DNA preparations on agarose gels, and exhibit the expected properties for circular extrachromosomal plasmids, i.e. they exist in three topological states: covalently closed, nicked circular and linear. In addition, multimeric forms can be readily detected (Dale 1981; Dale *et al.* 1981; Powling 1981; Abbott *et al.* 1985; Bendich 1985). It seems probable that these multimeric forms arise through the process of homologous recombination rather than as a result of replication.

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Linear plasmids associated with plant mitochondria

Linear plasmids have been identified in four species (table 2). The main characteristic features of these molecules are their terminal inverted repeats and a polypeptide covalently attached to the 5′—termini. In *Brassica* spp. and the S cytoplasm of maize, the abundance of the linear plasmids appears to be determined by the nuclear genotype (Laughnan *et al.* 1981; Kemble *et al.* 1986). The structure of the molecules, their linearity, and particularly the presence of terminal inverted repeats, prevents the formation of higher multimeric forms. The 11.3 kb plasmid of *Brassica* and the N1 and N2 plasmids of sorghum exhibit no sequence homology to their respective mitochondrial genomes or to the associated mitochondrial plasmids. This contrasts with the linear plasmids of *Zea* spp., which have homologous sequences in the mitochondrial genome. The best studied are the linear S1 and S2 plasmids from the S cytoplasm of maize. S1 and S2 can integrate into the mitochondrial genome. This integration occurs as a direct result of homology between the terminal inverted repeat sequences of S1 and S2 and the mitochondrial genome. Hybridization and sequencing studies have demonstrated that this homology involves the terminal 186 bp of the inverted repeat (Schardl *et al.* 1985). This 186 bp sequence in the mitochondrial genome forms part of a longer repeated sequence, designated R1*, which itself is derived from the related R1 plasmid (Levings *et al.* 1983; Houchins *et al.* 1986). Recombination between the terminal 186 bp of the inverted repeat of either of the S plasmids and the R1* repeat of the S mitochondrial genome leads to the generation of a large number of linear structures (Schardl *et al.* 1984, 1985). The main features of this recombination are illustrated in figure 1.

Protein covalently attached to DNA prevents its entry into agarose gels. Therefore, the linear plasmids or linear mtDNA structures with a terminal protein, whether digested with a restriction endonuclease or undigested, will not enter an agarose gel unless the protein is first removed. In the S mitochondrial genome, four unique sequences flank the R1* repeat. These

TABLE 2. LINEAR PLASMIDS OF PLANT MITOCHONDRIA

(ND, size of TIR not determined.)

		size/kb	TIR size/bp	5′-polypeptide	references
Brassica spp.		11.3	ND	yes	Palmer *et al.* (1983) Erickson *et al.* (1985)
Sorghum bicolor					
male-sterile accession IS1112C	N1	5.8	ND	yes	Chase & Pring (1986)
	N2	5.4	ND	yes	
Zea mays					
S cytoplasm	S1	6.4	208	yes	Levings & Sederoff (1983)
	S2	5.4	208	yes	Paillard *et al.* (1985)
	2.3L	2.3	175	yes	Bedinger *et al.* (1986)
	2.1L[a]	2.1	175	yes	
RU cytoplasm	R1	7.4	ND[b]	yes	Weissinger *et al.* (1982)
	R2	5.4	ND[b]	yes	
Zea diploperennis	D1	7.4	ND	yes	Timothy *et al.* (1983)
	D2	5.4	ND	yes	

[a] Identical to the 2.3L, apart from a small deletion.

[b] The TIR size has not been determined directly by sequencing, but sequencing of the integrated versions would suggest a size of 187 bp (Houchins *et al.* 1986).

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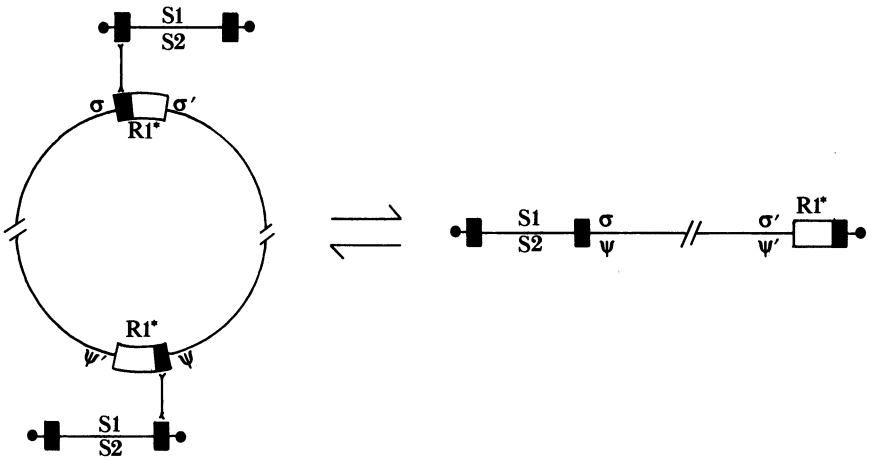

FIGURE 1. Linearization of the S-mitochondrial genome by S1 and S2. The S-mitochondrial genome contains a repeated DNA sequence, R1*, which has homology to the R1 plasmid and includes a sequence homologous to the first 186 bp of the terminal inverted repeats of the S plasmids. The four unique sequences flanking the two copies of the repeat have been designated σ, σ′, ψ and ψ′. The *cox*1 gene is located close to one copy of the repeat in the unique sequence designated σ. Recombination between R1* and either of the two terminal repeats of the S-plasmids gives linear molecules having S1 or S2 in either polarity at one end with the R1* sequence at the other. All the linear chromosomes have terminal proteins.

have been designated σ, σ′, ψ and ψ′ respectively. Hybridization and sequencing studies have demonstrated that the *cox*I gene can be identified with the σ sequence (Isaac *et al.* 1985; Schardl *et al.* 1985). By using *cox*I as a hybridization probe onto restriction digests of S mtDNA, additional hybridizing bands are detected after proteinase K treatment of the mtDNA (Schardl *et al.* 1984, 1985). However, little evidence for additional hybridizing fragments was obtained when mtDNA from RU cytoplasm of maize and *Zea diploperennis* (ZD) was similarly treated (data not shown). By using the clone of the 3680 bp *Bam*HI fragment, from the repeated DNA sequence adjacent to the R1 and R2 homologous sequences in the fertile cytoplasm, as a probe, evidence for additional fragments was obtained for RU and ZD mtDNA but not for S mtDNA (figure 2). This demonstrates that the R and D plasmids interact via recombination with the mitochondrial genome in a manner analogous to that of the S plasmids (figure 1), although the site of recombination between the R and D plasmids and the RU and ZD mitochondrial genomes is unrelated to the positions at which S1 and S2 recombine with the mitochondrial genome.

Interestingly, the sequence arrangement around the recombination site in the RU and ZD mitochondrial genomes more closely resembles the N than the S mitochondrial genome. This is particularly true for the RU mitochondrial genome, the restriction profile of which exhibits marked similarities to the restriction profile of the N mitochondrial genome. It is therefore possible that the RU cytoplasm is the direct progenitor of the current North American fertile (N) cytoplasms. The N cytoplasms no longer contain free replicating R plasmids but do contain integrated and defective copies (Houchins *et al.* 1986).

The structure of plant mitochondrial genomes

Restriction maps and the organization of the mitochondrial genomes of nine species have been described (table 3). Omitted from this list is the mitochondrial genome of wheat (Quetier

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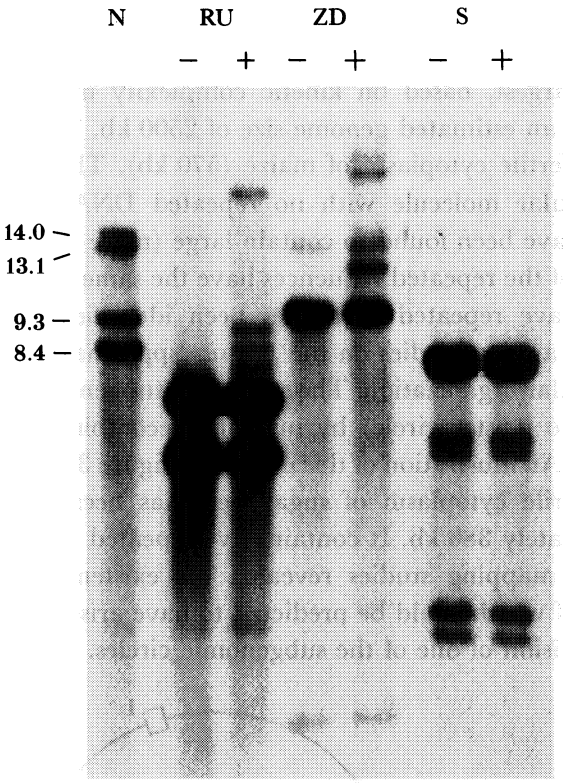

FIGURE 2. Evidence for linear mitochondrial chromosomes in the RU and ZD cytoplasms. Radioautograph of N, RU, ZD and S mtDNAs isolated without (−) or with (+) proteinase K digestion (500 μg ml^{-1} for 3 h in 0.5% (by mass) SDS before phenol extraction of the mitochondrial lysate). mtDNA was digested with *Xho*I and the fragments separated in a 1% agarose gel by electrophoresis. Restriction fragments were transferred to nitrocellulose and the filter was probed with the 3860 bp *Bam*HI fragment, which contains a part of the 5270 bp repeat in the Wf9-N maize mitochondrial genome (Houchins *et al.* 1986). Restriction-fragment sizes are given in kilobase pairs (kbp).

TABLE 3. PLANTS FOR WHICH RESTRICTION MAPS OF THE MITOCHONDRIAL GENOMES ARE COMPLETE

species		genome size	repeats		SGC[b]		
common name	Latin name	kb	no.	size/kb	size/kb		references[c]
white mustard	*Brassica hirta*	208	none	—	—		1
turnip	*Brassica campestris*	218	1	2	135	83	2
cabbage	*Brassica oleracea*	219	1	2	170	49	3
oilseed rape	*Brassica napus*	221	1	2	123	98	3
black mustard	*Brassica nigra*	231	1	7	135	96	4
radish	*Raphanus sativa*	242	1	10	139	103	4
spinach	*Spinacea oleracea*	327	1	6	234	93	5
sugar beet	*Beta vulgaris* (S)[d]	386	5	ND	many		6
maize	*Zea mays* (Wf9-N)	570	6	14,12,10, 5,2,1	many		7

[a] Repeat sizes are approximate and are based mainly on restriction mapping data.
[b] SGC, subgenomic circles.
[c] 1, Palmer & Herbon (1987*a*); 2, Palmer & Shields (1984); 3, Palmer & Herbon (1987*b*); 4, Palmer & Herbon (1986); 5, Stern & Palmer (1986); 6, T. Brears & D. M. Lonsdale (unpublished data); 7, Lonsdale *et al.* (1984).
[d] Sterile cytoplasm.

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et al. 1985, where uncertainty in the completeness of the physical map prevents a complete description of its organization. The genome of *Brassica hirta* is the smallest known mitochondrial genome (208 kb). The largest, based on kinetic complexity measurements, is muskmelon (*Cucumis melo*), which has an estimated genome size of 2500 kb. The largest mapped genome is that from the Wf9-N fertile cytoplasm of maize (570 kb). The mitochondrial genome of *B. hirta* is a simple circular molecule with no repeated DNA elements. All other plant mitochondrial genomes have been found to contain large (more than 200 bp) repeated DNA sequences. The majority of the repeated sequences have the same relative orientation. Only in sugar beet and maize have repeated sequences been identified which have an inverted orientation. Restriction mapping studies on the *Brassica* spp., maize and sugar beet genomes have revealed a multicircular organization. The smaller or subgenomic circles could be derived from one large circle (the master circle) by invoking recombination between the directly repeated DNA sequences. An illustration of this is given in figure 3. The mitochondrial genome from the Owen male-sterile cytoplasm of sugar beet has been determined, from cosmid mapping, to be approximately 386 kb. It contains five repeated sequences, four of which are directly orientated. The mapping studies revealed the existence of at least 6 additional subgenomic circles each of which could be predicted to have arisen from the master circle or, alternatively, from subdivision of one of the subgenomic circles.

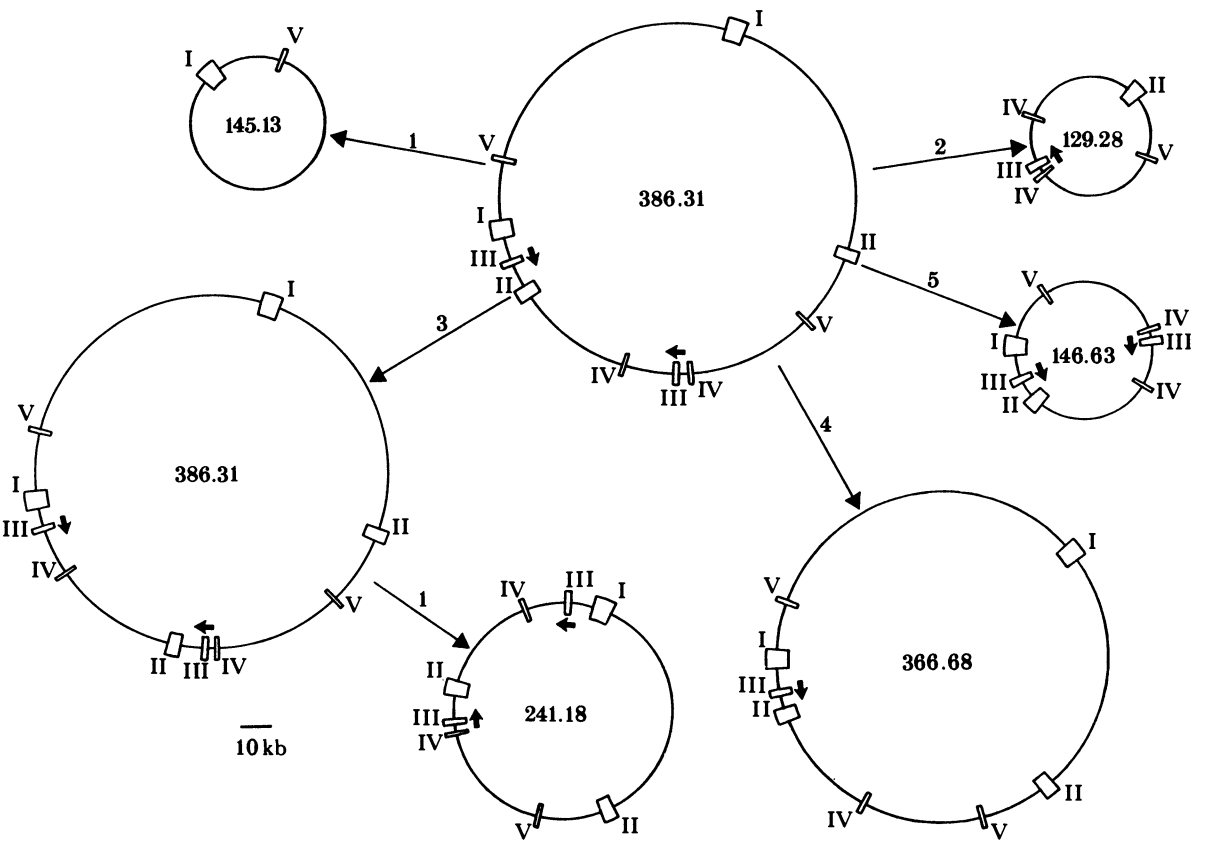

FIGURE 3. The mitochondrial genome of sugar beet. Physical mapping studies with overlapping cosmid clones have identified the circles illustrated. In addition to the two isomeric forms of master circle which result from recombination between the single pair of inverted repeats (III), recombination between the pairs of direct repeats leads to the formation of the smaller subgenomic circles. Many other circular forms arising from both intra- and intermolecular recombination events between repeats can be predicted but are not illustrated.

This complexity of the genome organization is reflected in the complexity of the restriction endonuclease profiles. The presence of submolar and supramolar bands correlates well with a complex multicircular organization resulting from recombination across repeated DNA elements.

Recombination and repeated DNA *sequences*

It is apparent from the analysis of the mitochondrial restriction profiles of *Brassica* spp., where the genome has a tripartite structure, that the four restriction fragments representing the repeated DNA element, and the four pairwise combinations of unique sequences that are located on the master circle and two subgenomic circles, are present in approximately equal stoichiometry (Palmer & Herbon 1986). Similar results are obtained by using hybridization probes for repeated DNA elements in wheat (Falconet *et al.* 1985), maize (Lonsdale *et al.* 1983) and other plants (Stern & Palmer 1984). An example is shown in figure 2, lane 1. The four hybridizing fragments each contain a single copy of the 5270 bp repeated DNA sequence and one of the four pairwise combinations of unique sequences which flank the repeated DNA sequence in the N mitochondrial genome of maize. In this example, the unique sequence combinations have been designated α-R1 (14.0 kb), α-R2 (13.0 kb), β-R1 (9.3 kb) and β-R2 (8.4 kb) (Lonsdale *et al.* 1983; Houchins *et al.* 1986).

Table 4. Sequenced repeats

species				
common name	Latin name	repeat[a]	size/bp	references[b]
maize	*Zea mays*	1	5270	1
		3	677	2
		S1/S2 TIR	208	3,4
		2.3 TIR	175	5
		*atp*6	122	6
		T-URF13/25	55	7
evening primrose	*Oenothera berteriana*	COXI/COXIII	657	8

[a] Repeated sequences of maize have been assigned numbers (Hodge & Lonsdale 1987) or have been identified by their association with particular plasmids or genes.

[b] 1, Houchins *et al.* (1986); 2, D. M. Lonsdale & P. G. Isaac (unpublished data); 3, Levings and Sederoff (1983); 4, Paillard *et al.* (1985); 5, Bedinger *et al.* (1986); 6, Dewey *et al.* (1985); 7, Rottmann *et al.* (1987); 8, Hiesel *et al.* (1987).

Several repeated DNA elements have been sequenced; these are listed in table 4. For those sequenced repeats, both copies of the repeat are identical and there is little apparent sequence homology between different repeats. Sequences known to promote site-specific recombination in other organisms can be found in some, but not all, of the repeats, although this kind of analysis is necessarily trivial in the absence of a functional test. Hybridization studies in *Brassica* spp. and in maize demonstrate that repeated DNA sequences in one species exist as single-copy DNA in others. This result strongly suggests that it is the sequence duplication itself that provides the opportunity for recombination to be observed, rather than there being sequences, embedded within the repeat, which promote recombination. Indeed all the observed complexities of mitochondrial genome organization in higher plants can be conveniently explained by invoking homologous recombination.

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Homologous recombination between circular plasmids would generate the plasmid multimers; it would also explain the recombination between plasmids and the main genome, where the main genome has sequences homologous to the plasmids, and the formation of the multipartite structure as a result of recombination across repeated DNA elements. It also predicts that the master circle and all the subgenomic circular species can form higher multimers by intermolecular recombination. Direct evidence for this has been obtained in tobacco (Dale *et al.* 1983), where one of the small subgenomic circles of 10.1 kb has been shown by restriction mapping to be present as a circular dimer and trimer. The monomer sequence has also been shown to exist with other unrelated sequences in a larger circular form.

Mitochondrial genome organization cannot be visualized as a rigid structure but rather must be seen as a dynamic system where more than just one form of the genome exists. The rates of inter- and intramolecular recombination will be determined by the length of the homologous sequences interacting, and by their sequence composition (de Zamaroczy *et al.* 1983). The final stoichiometries will be dependent on the rates at which sequences have the opportunity to recombine relative to the rates of replication of the individual molecules.

The above discussion considers recombination between homologous sequences whether they are on the same or separate molecules and where the products of recombination are easily identifiable. There are instances, however, where recombination happens infrequently and is not easily detected; an example is the formation of petite genotypes in wild-type populations of yeast mtDNA (Clark-Walker *et al.* 1981). Similar low-frequency recombination events can be detected in mitochondrial genomes of higher plants. In the four maize cytoplasms, four genomic arrangements of the *atp*A gene have been detected by Small *et al.* (1987) using the restriction endonuclease *Bam*HI. The normal fertile cytoplasm (N) has arrangements 1 and 2; the C, S and T cytoplasms have arrangements 1, 3 and 4 respectively. It was also demonstrated that different maternal lineages of the N and S cytoplasm types have, in addition to the expected arrangement of the *atp*A gene, substoichiometric restriction fragments that are equivalent to the other arrangements. In fact, in some maternal lineages these substoichiometric fragments have been amplified, either replacing the original fragment or being present in addition to it, thereby creating a sequence duplication such as exists in some of the N mitochondrial genomes (Lonsdale *et al.* 1984). Similar substoichiometric fragments can be detected in fertile revertants of the male-sterile S cytoplasm (figure 4). In the fertile revertant, R274, the strongly hybridizing fragments (arrowed) are the minor hybridizing fragments of R285, R296 and R369 and *vice versa*. It appears that, during the events of fertility reversion, the mitochondrial genome has reorganized and that the reorganization can take one of several routes. Reorganization is probably initiated by recombination between small repeated DNA sequences; this generates a novel genome organization and it is this form of the genome which is selected and eventually predominates. The spatial distribution of these small repeated sequences within any genome will dictate the ways mitochondrial genomes will evolve structurally.

There are duplicated sequences between which recombination cannot be detected directly because the frequency of recombination is too low. Examples are the 122 bp *atp*6 repeat (Dewey *et al.* 1985) and the T-URF13/25 repeat (Rottmann *et al.* 1987). In the latter example, although recombination between the two copies of the repeat cannot be detected directly in mtDNA by probing, analysis of fertile revertants of the male-sterile T cytoplasm of maize demonstrated that recombination between the two copies of the T-URF13/25 repeat must

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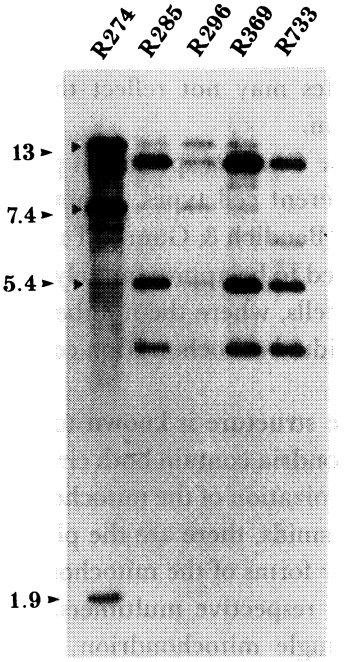

FIGURE 4. Detection of substoichiometric restriction fragments in the mtDNA of fertile revertants from the male-sterile VgS cytoplasm of maize. Total mtDNA was prepared from the fertile revertants R274, R285, R296 and R369 and R733. It was digested with *Xho*I and the fragments were separated in a 1% agarose gel by electrophoresis. Restriction fragments were transferred to nitrocellulose and probed with pGSSIG1 (Schardl *et al.* 1985), which contained sequences of σ and the R1* repeat (see figure 1). The restriction fragments showing marked alteration in hybridization intensity between revertants are arrowed.

have occurred in order to delete a mitochondrial gene (Rottmann *et al.* 1987) and give rise to a new mitochondrial genome organization. Interestingly, this organization reflects that of the N mitochondrial genome at this locus (Abbott & Fauron 1986). This is analogous to rearrangements observed directly for the *atp*A gene (Small *et al.* 1987). Other non-reciprocal recombination events occur in the S fertile revertants, involving short homologous sequences in the terminal inverted repeat of the S2 plasmid and the mitochondrial genome with high molecular mass (Schardl *et al.* 1985); in *Oenothera*, a rare and non-reciprocal recombination event led to the formation of subgenomic circle 3 (Manna Brennicke 1986).

Intra- and intermolecular recombination, involving both reciprocal and non-reciprocal events, and the selection and maintenance of these arrangements in different maternal lineages, confirm that homologous recombination is a frequent process in plant mitochondria.

The mtDNA *content of mitochondria*

The number of mitochondria varies considerably between different cell types; a review of this literature is given in Bendich & Gauriloff (1984). There are, however, few critical studies relating the mtDNA content to the number of mitochondria in cells or in specific tissues. One of the earliest studies was by Suyama & Bonner (1966), who isolated and counted the mitochondria before isolating mtDNA. By relating the amount of DNA obtained to the number of mitochondria, a value of 5×10^{-10} μg DNA per mitochondrion in mung bean (*Phaseolus aureus*), turnip (*Brassica rapa*), sweet potato (*Ipomoea batatas*) and onion (*Allium cepa*) was obtained. If the size of the turnip mitochondrial genome is assumed to be 220 kb (Palmer &

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Shields 1984), 5×10^{10} μg is equivalent to two genomes or one master circle and two subgenomic circles per mitochondrion. This result is perhaps quite fortuitous, as the number of 'mitochondria' isolated as vesicles may not reflect the actual number of mitochondria present in the tissue prior to isolation.

Electron microscopy studies on the *Cucurbita* species, in particular, revealed a wide range in the number of mitochondria in different cell types, although the ratio between nuclear and mtDNA remained roughly constant (Bendich & Gauriloff 1984). The number of mitochondrial genomes per diploid cell was estimated to be approximately 110–140 (Ward *et al.* 1981). It was therefore evident that in cotyledon cells, where the number of mitochondria was estimated to be greater than 10^3 per cell, an individual mitochondrion could not possibly contain a complete genome.

Where the mitochondrial genome structure is known to be complex, for example in maize and in sugar beet; where the mitochondria contain both circular and linear DNA plasmids, e.g. in the S cytoplasm of maize, the organization of the mitochondrial genome is extremely diverse. In addition to the small circular plasmids, there are the plasmid multimers, the linear S1 and S2 plasmids and the numerous linear forms of the mitochondrial genome as well as the master circle, subgenomic circles and their respective multimers. To envisage such a complex and diverse genome arising within a single mitochondrion, which may not contain an entire genome or even which contains only a few genomes, is an impossibility.

The resolution of this conundrum is to assume that, as in yeast (Stevens 1981), the mitochondria of cells of higher plants form a dynamic syncytium. The mtDNA population of individual cells would therefore be panmictic and in a state of recombinational equilibrium (see Dujon 1981). Direct evident to support mitochondrial fusion and active mtDNA recombination is available from cell fusion studies (for a review, see Lonsdale (1987)). Fusing cells from different species of tobacco (*Nicotiana*), *Petunia* and *Brassica*, where the organelle genomes are recognizably different based on restriction-profile analysis, leads to chimeric cytoplasms where both parental organelle populations are equally represented. The subsequent analysis of both the mitochondrial and the chloroplast genomes in callus or plant material derived from single fusion cells demonstrates that organelle sorting out has occurred and that, with few reported exceptions, the mtDNA restriction profile is novel, exhibiting restriction fragments from both parental cell lines in addition to having completely new restriction fragments. This demonstrates that interparental mitochondrial fusion and mtDNA recombination must have occurred. This is not generally true for the chloroplast genomes, where the chloroplast population of fusion hybrids is derived from either one of the two parents. Only a single case of chloroplast recombination in higher plants has been reported and described (Medgyesy *et al.* 1985), although chloroplast fusion and ctDNA recombination has been reported in the alga *Chlamydomonas reinhardtii* (Lemieux & Lee 1987).

Inheritance and evolution of the cytoplasmic organelle genomes

The chloroplast and mitochondrion are structurally and functionally different. Moreover, mitochondria can fuse, thus allowing the genomes to mix and recombine readily. This appears not to be the case for chloroplasts, where fusion (based on the identification of novel recombinant chloroplast genomes) is rare. The two organelles therefore behave entirely differently within the cell; it is this basic difference between the organelles that must explain the way in which the organization of the genomes evolves and the observed differences in the rates of base-pair mutations.

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Indeed, at the DNA sequence level, chloroplast genomes are evolving many times faster than mitochondrial genomes (for review, see Palmer (1985) and Palmer & Herbon (1987*b*)). This is simply illustrated by comparing the wheat chloroplast $tRNA^{Pro}$ and $tRNA^{Trp}$ intergenic region with the tobacco and liverwort (*Marchantia*) chloroplast sequences and with the same sequence which is present in the mitochondrial genomes of wheat and maize (Shinozaki *et al.* 1986; Ohyama *et al.* 1986; Marechal *et al.* 1987). The intergenic-region sequences in the mitochondrial genomes of wheat and maize show only 7% sequence divergence, whereas a comparison of the wheat chloroplast and wheat mitochondrial sequences show a considerable degree of divergence (over 50%). This degree of sequence divergence in the $tRNA^{Pro}$–$tRNA^{Trp}$ intergenic region is also seen in comparisons of different chloroplast genomes. What is evident is that the chloroplast sequence present in the mitochondrial genomes of wheat and maize is an evolutionary relic, reflecting the sequence of this region of the chloroplast genome at the time it was transferred into the mitochondrial genome before the evolutionary divergence of wheat and maize. The rate of sequence evolution between mitochondrial and chloroplast genomes is markedly different. However, chloroplast genome structure and sequence organization is remarkably stable, being similar in many groups of the photosynthetic eukaryotes. This contrasts with mitochondrial genome structure and sequence organization, which varies markedly even between closely related species. So it is important, in considering the evolution of the chloroplast and mitochondrial genomes at both the structural and base-sequence levels, to identify the factor or factors which cause the marked differences in evolutionary rates between the organelles within a cytoplasm.

The noticeable difference in the behaviour of the organelles is in the frequency of organelle fusion (based on observed intergenomic recombinants). This must be the major determinant influencing not only the structural evolution of the genomes but also the base sequence evolution. The chloroplast has, like the mitochondrion, a highly active recombination system that promotes recombination between the inverted repeats and produces two isomeric forms of the genome (Palmer 1985; Brears *et al.* 1986). It would seem probable that, as in the mitochondrion, homologous recombination occurs, functioning both intra- and intermolecularly on the chloroplast DNA population within any given chloroplast. If the chloroplast DNA population within individual chloroplasts is restricted, particularly during gamete formation, then the opportunities for novel chloroplast genome organizations to arise will be limited. The majority of the recombination both between and within molecules will be channelled through the large inverted repeats. Copy correction after intermolecular recombination will occur, but the inability of chloroplasts within any cell to fuse will allow individual chloroplasts to accumulate different base-sequence changes rapidly. Each chloroplast, therefore, has the opportunity to contribute to the identity of the chloroplast DNA (ctDNA) population in subsequent generations.

The mitochondrion is not a discrete organelle, as is the chloroplast. The chondriome and the mtDNA population of the cell must be considered as single entities. It is the behaviour of these that allows the rapid structural evolution of mitochondrial genomes yet suppresses the rate of base-sequence evolution. If individual cells contained relatively large numbers of mitochondrial genomes, and mtDNA recombination and copy correction occurred many times within a cell generation and more frequently than mtDNA replication, then it would be unlikely that single-base mutations would become fixed in the population. The mitochondrial genome organization, on the other hand, would be able to evolve rapidly. If only part of the chondriome at each cell division is inherited, then there is a possibility that one of the two

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daughter cells may not inherit a complete genome. Although this is perhaps unlikely, such a situation would be lethal. However, if a complete genome were not stoichiometrically represented in one of the daughter cells, perhaps with one of the rare substoichiometric circular species completing the genetic complement, then there would be a strong selection pressure for the mitochondrial genome to accommodate this normally rare sequence arrangement to maintain cell viability. This situation would promote genome reorganization. Mitochondrial genome reorganizations are observed in the fertile revertants of the S and T male-sterile cytoplasms of maize and in plant material regenerated from fused protoplasts. Implicit in this is the prediction that any genomic rearrangement occurring has a finite chance of being established in identical or modified form in the equilibrium population. The lack of functional constraints imposed by large mitochondrial genomes provides little selection against the establishment of novel organizations of mtDNA; this situation is observed in a large array of very different mitochondrial genome organizations in different lineages of single species (Small *et al.* 1987) as well as in different species (see table 3).

Concluding remarks

The mitochondria and chloroplasts of photosynthetic eukaryotes are demonstrably unique organelles, however they are considered. Genome organization, gene structure and the control of gene expression are areas where considerable research efforts have led to an understanding of the overall functioning of the organelle genome. The interactions between organelles and their modes of inheritance, particularly in higher plants, are aspects of organelle biology that have not been addressed in any detail and which are necessary if organelle inheritance and evolution are to be fully understood.

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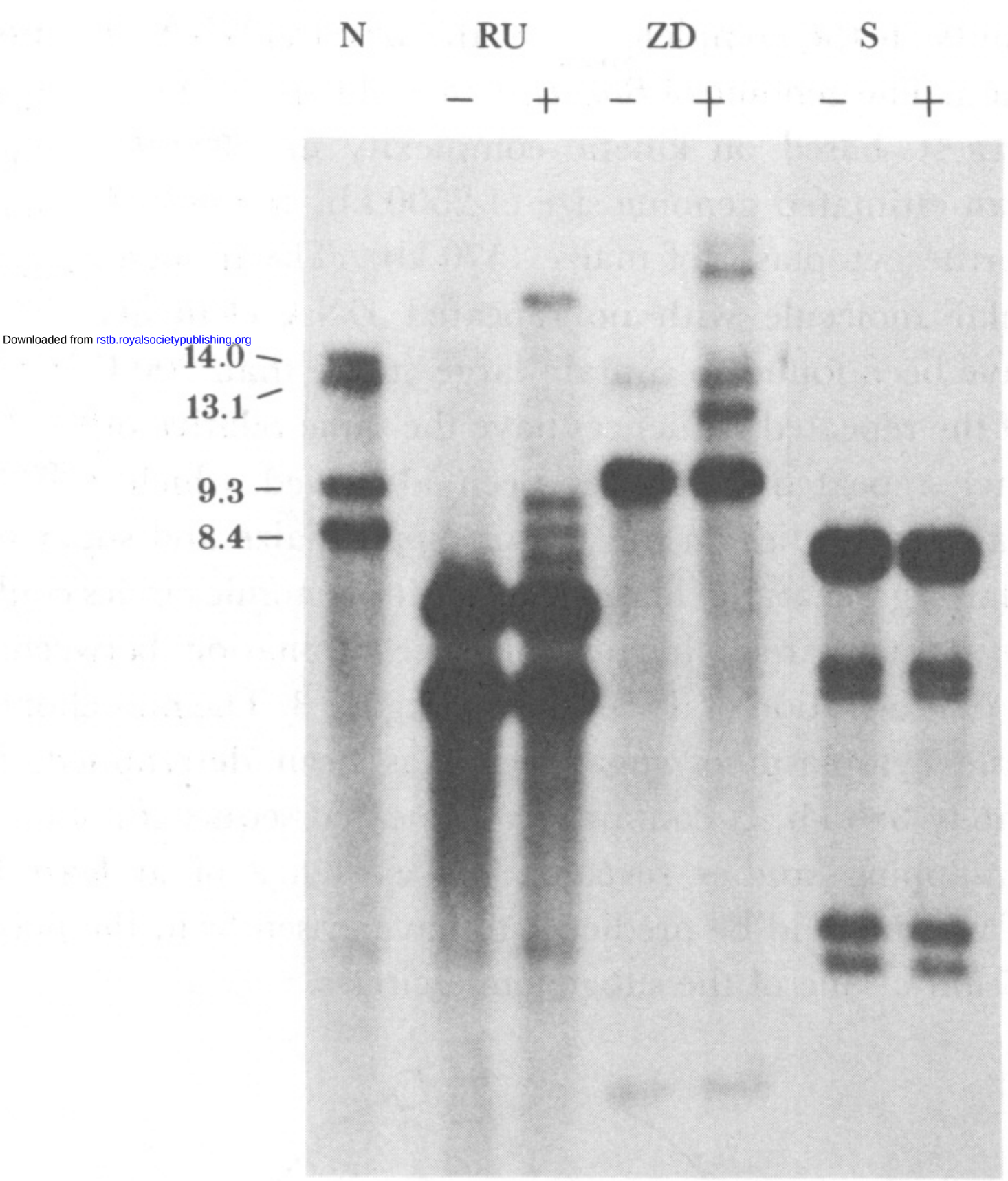

FIGURE 2. Evidence for linear mitochondrial chromosomes in the RU and ZD cytoplasms. Radioautograph of N, RU, ZD and S mtDNAs isolated without (−) or with (+) proteinase K digestion (500 μg ml^{-1} for 3 h in 0.5% (by mass) SDS before phenol extraction of the mitochondrial lysate). mtDNA was digested with *Xho*I and the fragments separated in a 1% agarose gel by electrophoresis. Restriction fragments were transferred to nitrocellulose and the filter was probed with the 3860 bp *Bam*HI fragment, which contains a part of the 5270 bp repeat in the Wf9-N maize mitochondrial genome (Houchins *et al.* 1986). Restriction-fragment sizes are given in kilobase pairs (kbp).

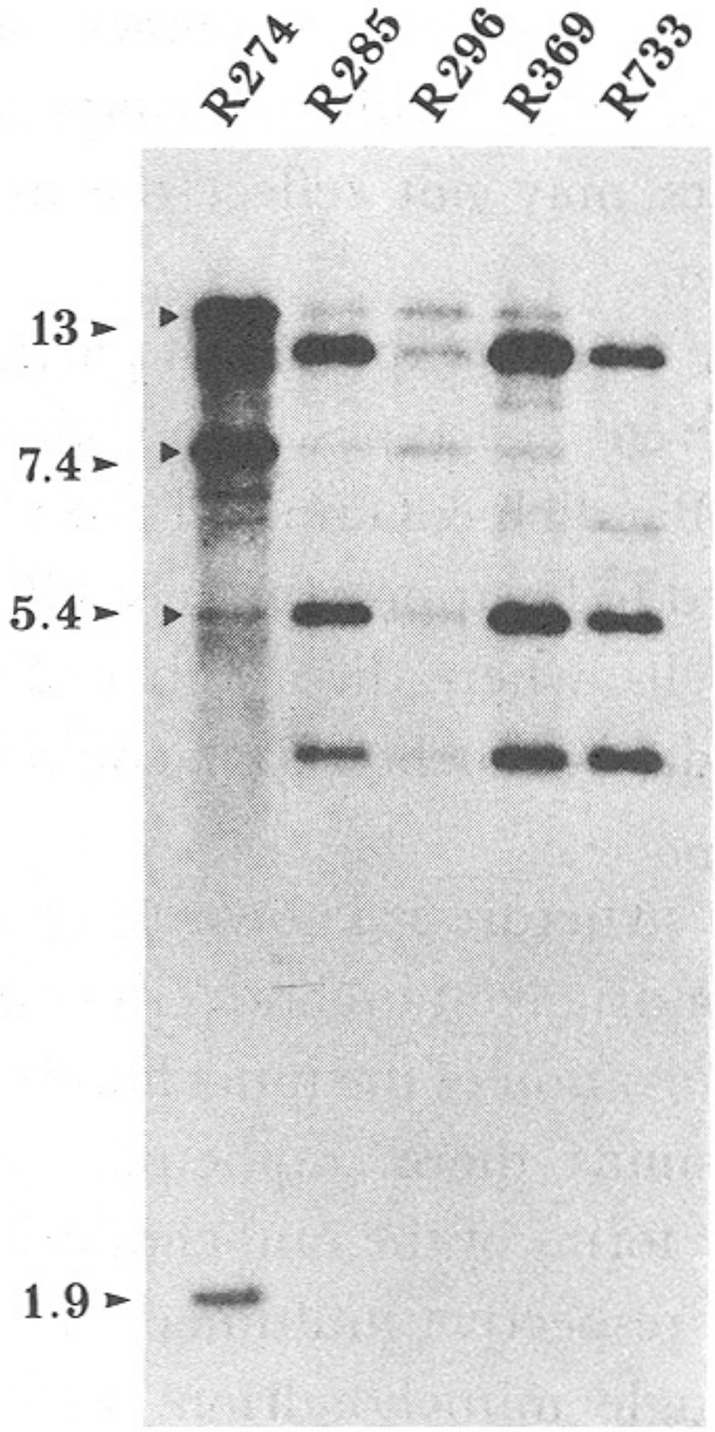

FIGURE 4. Detection of substoichiometric restriction fragments in the mtDNA of fertile revertants from the male-sterile VgS cytoplasm of maize. Total mtDNA was prepared from the fertile revertants R274, R285, R296 and R369 and R733. It was digested with *Xho*I and the fragments were separated in a 1% agarose gel by electrophoresis. Restriction fragments were transferred to nitrocellulose and probed with pGSSIG1 (Schardl *et al.* 1985), which contained sequences of σ and the R1* repeat (see figure 1). The restriction fragments showing marked alteration in hybridization intensity between revertants are arrowed.