Theor Appl Genet (1991) 81:420–427

THEORETICAL AND APPLIED GENETICS

Comparison of the organization of the mitochondrial genome in tomato somatic hybrids and cybrids

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Received April 24, 1990; Accepted August 22, 1990
Communicated by P. Maliga

Summary. The organization of the mitochondrial genome in somatic hybrids and cybrids regenerated following fusion of protoplasts from cultivated tomato, *Lycopersicon esculentum,* and the wild species, *L. pennellii,* was compared to assess the role of the nuclear genotype on the inheritance of organellar genomes. No organellar-encoded traits were required for the recorvery of either somatic hybrids or cybrids. The organization of the mitochondrial genome was characterized using Southern hybridization of restriction digestions of total DNA isolated from ten cybrids and ten somatic hybrids. A bank of cosmid clones carrying tomato mitochondrial DNA was used as probes, as well as a putative repeated sequence from *L. pennellii* mitochondrial DNA. The seven cosmids used to characterize the mitochondrial genomes are predicted to encompass at least 60% of the genome. The frequency of nonparental organizations of the mitochondrial genome was highest with a probe derived from a putative repeat element from the *L. pennellii* mitochondrial DNA. There was no difference in the average frequency of rearranged mitochondrial sequences in somatic hybrids (12%) versus cybrids (10%), although there were individual cybrids with a very high frequency of novel fragments (30%). The frequency of tomato-specific mtDNA sequences was higher in cybrids (25%) versus somatic hybrids (12%), suggesting a nuclear-cytoplasmic interaction on the inheritance of tomato mitochondrial sequences.

Key words: Tomato – Mitchondrial DNA – Somatic hybrids – Cybrids

Introduction

Frequently, following protoplast fusion, the nuclear and mitchondrial organelles fuse and produce hybrid genotypes (review Galun and Aviv 1983). Plastid organelles rarely fuse to result in hybrid genotypes (Medgyesy et al. 1985; Thanh and Medgyesy 1989); instead, one or the other parental genotype predominates in the regenerated fusion product, presumably the result of sorting of the parental organelles during cell division in the callus or microcallus stage. The fate of the mitochondrial and nuclear genomes following fusion is more complex. Depending on the relatedness of the fusion partners, the nuclear genomes may be stably inherited in the regenerated fusion products, or the genetic information of one of the parents may be preferentially lost. Tetraploid somatic hybrids can be recovered in intrageneric fusions; loss of genetic information is more frequent with intertribal or wider fusion combinations (Dudits et al. 1980).

Analysis of the genotype of the mtDNA in fusion products is complicated by the dynamic nature of the mitochondrial genome. Higher plant mitochondrial genomes are relatively large, 200–2,400 kbp (Ward et al. 1981), and multipartite (Palmer and Shields 1984; Lonsdale et al. 1983). The circular elements of the mitochondrial genome can recombine through direct and indirect repeat sequences to create a complex organization of subgenomic species in varying stoichiometries. Subgenomic molecules present in very low amounts have been named sublimons (Small et al. 1987). Further complicating the characterization of the mtDNA in fusion products is the effect of somaclonal variation. Alterations in the organization of plant mtDNA have been induced by tissue culture (Brears et al. 1989; Hartmann et al. 1989; McNay et al. 1984; Shirzadegan et al. 1989).

Mitchondrial genomes of the fusion partners may be inherited in a manner similar to that of the chloroplast, i.e., one parental genotype may predominate. This has been observed in interspecific fusions in *Brassica* (Kemble et al. 1988; Jourdan et al. 1989), as well as in fusions between tomato and potato (Shepard et al. 1983). More frequently, fusion products contain mitochondrial

Table 1. Description of the tomato somatic hybrids and cybrids. The protoplasts used as fusion partners are listed; UC82-iodo indicates that the protoplasts were treated with iodoacetamide prior to fusion, *L. penn*-100 k, -3 k indicates that the protoplasts were exposed to 100 k rad or 3 k rad of radiation prior to fusion. The genotypes are designated E for *L. esculentum*, P for *L. pennelli*, and H for heterozygous; the number in parentheses indicates the number of loci scored

Cybrids	Fusion partners	Chromosome no.	Chloroplast genotype	Nuclear genotype
81	UC82-iodo + *L. penn*-100 k	24	E	E (2)
92A	UC82-iodo + *L. penn*-100 k	24	E	E (11)
92B	UC82-iodo + *L. penn*-100 k	24	E	E (8)
100A	UC82-iodo + *L. penn*-100 k	24	E	E (11)
100B	UC82-iodo + *L. penn*-100 k	24	E	E (–)
100C	UC82-iodo + *L. penn*-100 k		E	E (–)
100D	UC82-iodo + *L. penn*-100 k	24	E	E (–)
100E	UC82-iodo + *L. penn*-100 k	24	E	E (–)
121	UC82-iodo + *L. penn*-100 k	24	E	E (5)
122	UC82-iodo + *L. penn*-100 k	24	E	E (11)
Somatic hybrids				
1	Early Girl + *L. penn*	–	E	H (2)
2	Early Girl + *L. penn*	68	E + P	H (5)
3A	Early Girl + *L. penn*	54	E	H (5)
3B	Early Girl + *L. penn*	–	E	H (5)
3C	Early Girl + *L. penn*	–	E	H (5)
4	UC82 + *L. penn*	–	E	H (2)
5	UC82 + *L. penn*	–	E	H (2)
6	UC82 + *L. penn*-3 k	–	E	H (5)
7	UC82 + *L. penn*-3 k	44	E	H (5)
8	Early Girl + *L. penn*	–	P	H (5)

genomes that are apparently the result of recombination between the two parental genomes (Belliard et al. 1979; Boeshore et al. 1983; Chetrit et al. 1985; Jourdan et al. 1989; Bonnema et al. 1990). MtDNA restriction fragment length polymorphisms (RFLPs) for each fusion parent as well as novel fragments can be detected in a single regenerant. These novel fragments may be the result of either intergenomic recombination (Rothenberg et al. 1985) or sublimons.

The organization of the mitochondrial genome in plants can be affected by the nucleus (Laughnan and Gabay-Laughnan 1985). Characterization of the structure of the mtDNA in plant somatic hybrids and cybrids presents an approach to systematically investigate the effects of particular nuclear genotypes on the organization of the mitochondrial genome. While the mtDNA in cybrids and somatic hybrids has been described for a number of different plants, a comparison of the organization of the mtDNA present in fused cytoplasm with controlled differences in nuclear genotype has not been presented. Tomato somatic hybrids and cybrids formed following fusion of tomato cultivars with *Lycopersicon pennellii* have been described (O'Connell and Hanson 1987; Bonnema et al. 1990). These fusion products were recovered without the use of selectable traits encoded in organellar genomes, i.e., streptomycin resistance, albinism. This report describes the comparative organizations of the mtDNA in tomato somatic hybrids and cybrids.

Materials and methods

Plant material

The construction and initial characterization of the somatic hybrids between tomato and *L. pennellii* were described previously (O'Connell and Hanson 1987). The construction and initial characterization of the cybrids between tomato and *L. pennellii* have been described (Bonnema et al. 1990). The genotypes of the individual somatic hybrids and cybrids used in this study are listed in Table 1. In both fusion experiments, leaf mesophyll cells were the source of the tomato protoplasts and a suspension cell culture was the source of the *L. pennellii* protoplasts.

DNA isolation and Southern hybridizations

Total DNA was isolated from the somatic hybrids and cybrids as described by Bonnema et al. (1990). DNA was restricted with an excess of the indicated endonuclease for at least 3 h at 37 °C. The restricted DNA was electrophoresed through a 0.8% agarose gel, stained with ethidium bromide, photographed, denatured, and transferred to Zetabind nylon membrane. The conditions for hybridization and washing were essentially as described by O'Connell and Hanson (1987); the membranes were hybridized with probes oligolabelled with [^{32}P]-dCTP (Feinberg and Vogelstein 1983).

Purified chloroplast DNA was isolated from tomato leaf tissue as described by Saltz and Beckmann (1981). Purified mitochondrial DNA was isolated from either tomato leaf tissue or suspensions cells of *L. pennellii*, as described by Hanson et al. (1986).

Description of probes

Tomato mtDNA was partially digested with Mbo1 and cloned into the BamH1 site on the cosmid vector pHC79 (Hohn and Collins 1980). Individual recombinants were isolated and characterized. A description of the cosmid clones used in this study is provided in Table 2. Plasmid 2D4 contains a 2.1-kb Sal fragment cloned from *L. pennellii* mtDNA (McClean and Hanson 1986). Purified tomato cpDNA was restricted with Sal1 and the fragments were cloned into pUC8. A recombinant clone carrying the 27-kb Sal cpDNA fragment was identified by screening restriction digest of DNA minipreps of individual clones.

Results and discussion

Characterization of tomato mitochondrial cosmid clones

A cosmid library of tomato mtDNA was constructed. From this library, seven cosmid clones were selected for analysis of the mtDNA in the tomato somatic hybrids and cybrids. A description of these cosmid clones is presented in Table 2. The insert size of the cosmids ranged from 35 to 42 kb, with an average size of 38.3 kbp. Using heterologous probes, the presence of several mitochondrial genes have been assigned to the cosmids. The genes for the ATPase subunits α, 6, and 9 have been identified using heterologous probes form corn (Braun and Levings 1985; Dewey et al. 1985) and petunia (Young et al. 1986). The genes for the ribosomal RNAs 18S+5S and 26S (Chao et al. 1984; Stern et al. 1982), and cytochrome oxidase subunit II (Fox and Leaver 1981) were identified using heterologous probes from corn. The gene identified in Table 2 as '3.2 kb repeat' is an EcoRI fragment identified by us to be present in at least three different organizations of the tomato mitochondrial genome (data not shown).

Restriction mapping of the cosmid clones and cross hybridizations of Southern blots of restricted cosmid DNA were used to determine the extent of overlap in sequence of the seven cosmid clones (data not shown). Only two of the clones showed an appreciable overlap in sequence, A2 and B1; the five other cosmid clones – B3, D9, A1, A3, and C3 – carried unique sequences. Tomato cpDNA and mtDNA were also included on these Southern blots. While there was hybridization between some of the cosmids and cpDNA, none of the cosmid clones contained fragments that comigrated with cpDNA. The total mtDNA sequence estimated to be characterized by these seven cosmid clones was at least 230 kb.

The size of the tomato mitochondrial genome has been estimated to be 250 kb (Hause et al. 1986) and 350 kb (McClean and Hanson 1986). Both estimates are based on summation of restriction fragments observed in ethidium-bromide-stained gels of restricted mtDNA. This technique is expected to give an underestimate based on the complexity of the digestion. Our results in characterizing cosmid clones and phage clones carrying tomato mtDNA suggest that a genome size estimate of 250 kb is an underestimation. The genome size is probable in the range of 350 to 400 kb. Using this larger size estimate, screening 230 kb of the genome with the cosmid probes should permit characterization of at least 60% of the total genome.

Table 2. Description of the cosmid clones used to characterize the mtDNA. The insert size of the cosmids was determined from restriction mapping, the presence of mitochondrial genes was determined by Southern hybridization with heterologous genes, and the total number of parental-specific fragments identified with each cosmid clone using four restriction enzymes – BamH1, Sma1, Sal1, and HindIII – was determined for tomato (E) and *L. pennellii* (P)

Cosmid	Insert size (kbp)	mt Genes	No. of parental fragments	
			E	P
A1	38.6	26S, ATPase 9	2	2
A2	39.1	18S+5S, ATPase α, 6	3	2
A3	36.0	3.2 kb "repeat"	4	8
B1	35.1	ATPase 6, Cox II	2	2
B3	35.6	18S+5S	6	9
C3	42.0	26S	7	9
D9	41.8	ATPase 9	3	8

Determination of mtDNA RFLPs

To identify useful RFLPs, total DNA isolated from 'UC82' or *L. pennellii* was restricted with four different endonucleases – BamH1, Sma1, Sal1, HindIII – and replicate Southern blots were prepared. These blots were probed with a number of cosmids, and examples of these analyses are shown in Fig. 1. The seven cosmids used in the characterization of the mtDNA in fusion products were all shown to identify RFLPs between the two parental species (Table 2). On average, 2.4 RFLPs were observed per cosmid per restriction digestion. For example, of the four enzymes tested with the probe cosmid A2, two revealed polymorphisms (Fig. 1). There were no differences between the two species when the DNA was cut with Sal1 or HindIII, three differences when the DNA was cut with BamH1, and two differences when the DNA was cut with Sma1.

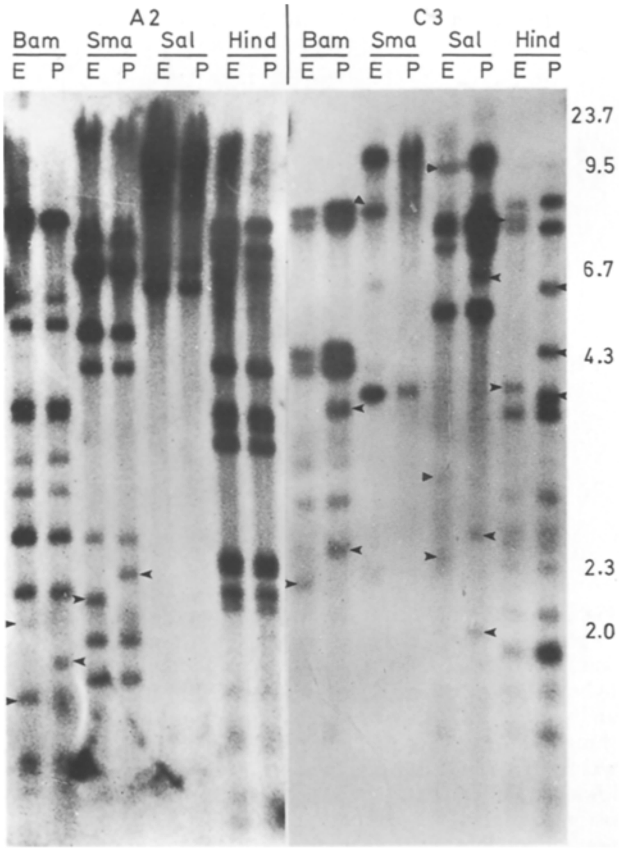

Fig. 1. Comparison of the similarity of the two parental mitochondrial genomes. Total DNA was isolated from leaf tissue of either UC82 (E) or *L. pennellii* (P), restricted with the indicated endonucleases, and Southern blots were prepared following electrophoresis. The blots were probed with oligolabelled cosmid clones, A2 or C3. The position of lambda DNA markers is indicated in kilobases, the presence of species-specific RFLPs is noted by *arrowheads*

Description of the nuclear and chloroplast genotypes of the regenerants

The individuals listed as somatic hybrids in Table 1 were characterized to be hybrid based on heterozygous isozyme banding patterns for at least two activities located on two different chromosomes (O'Connell and Hanson 1987). In addition, the morphological appearance of the regenerated plants was intermediate between the two parental plants. Three different tomato cultivars were used in the construction of the somatic hybrids, 'Early Girl,' 'Manapal,' and 'UC82.' No differences were ever observed in the somatic hybrids that could be attributed to the different cultivar sources. Two different tomato cultivars are represented in the sampling of the somatic hybrids used in this comparison (Table 1). The individuals listed as cybrids in Table 1 were characterized to have only tomato nuclear alleles at two or more loci (Bonnema et al. 1990). In addition, the appearance of the regenerated cybrids was similar to the 'UC82' parent, and the regenerated cybrids did not display any specific *L. pennellii* morphological characters.

The chloroplast genotype in all the regenerants was determined using a cloned fragment of tomato chloroplast DNA, the 27-kb Sal fragment present in the inverted repeat region of the chloroplast genome (Phillips 1985). There is a species-specific polymorphism in this region of the genome that can be detected using a HindIII digestion (Palmer and Zamir 1982). Total DNA isolated from the regenerants was restricted with HindIII, and a Southern blot of the digested DNA was probed with the 27-kb Sal fragment of tomato cpDNA. Based on the species-specific RFLPs, a chloroplast genotype was assigned to each regenerant and the results are listed in Table 1. All of the cybrids inherited the cpDNA of the tomato parent (Bonnema et al. 1990; Table 1), whereas some of the somatic hybrids inherited the cpDNA of the *L. pennellii* parent (Table 1). In one case, somatic hybrid no. 2, both of the parental cpDNAs were present. The plant tissue that was used for the DNA isolation of the somatic hybrids had been vegetatively propagated for at least 3 years from the time of the fusion. It is remarkable that the two chloroplast genotypes have persisted in equal abundance in somatic hybrid no. 2 for such a long period of time; the heterozygous state of the chloroplasts in this somatic hybrid is apparently stable.

Analysis of the mitochondrial DNA in tomato somatic hybrids

The total DNA isolated from tomato somatic hybrids formed between several different cultivars and the wild species *L. pennellii* was restricted with a variety of endonucleases, and Southern blots of these digests were probed with cloned fragments of tomato mtDNA. Examples of the autoradiograms obtained by this method are provided in Fig. 2 and 3, and a summary of the results obtained with seven cosmid clones and two plasmid clones, 2D4 and a clone carrying only the tomato 18S + 5S mitochondrial ribosomal RNA genes (Estabrook and O'Connell 1986) is listed in Table 3.

Cosmid clone A2 hybridizes to two tomato-specific BamH1 fragments, 1.8 and 1.0 kbp, and one *L. pennellii*-specific BamH1 fragment, 1.5 kbp (Fig. 2). The organization of the homologous mtDNA sequences in somatic hybrids 2, 4, 6, 7, and 8 is identical to the *L. pennellii* parent (Fig. 2). In somatic hybrids 3A, 3B, 3C, and 5, only one of the two tomato-specific fragments was observed. In somatic hybrid 1, a summation of both parental-specific fragments, 1.8, 1.5, and 1.0 kbp, was detected (Fig. 2). No novel nonparental bands were identified with this probe.

When DNA from *L. pennellii* is probed with plasmid clone 2D4, three *L. pennellii*-specific Sal1 fragments can

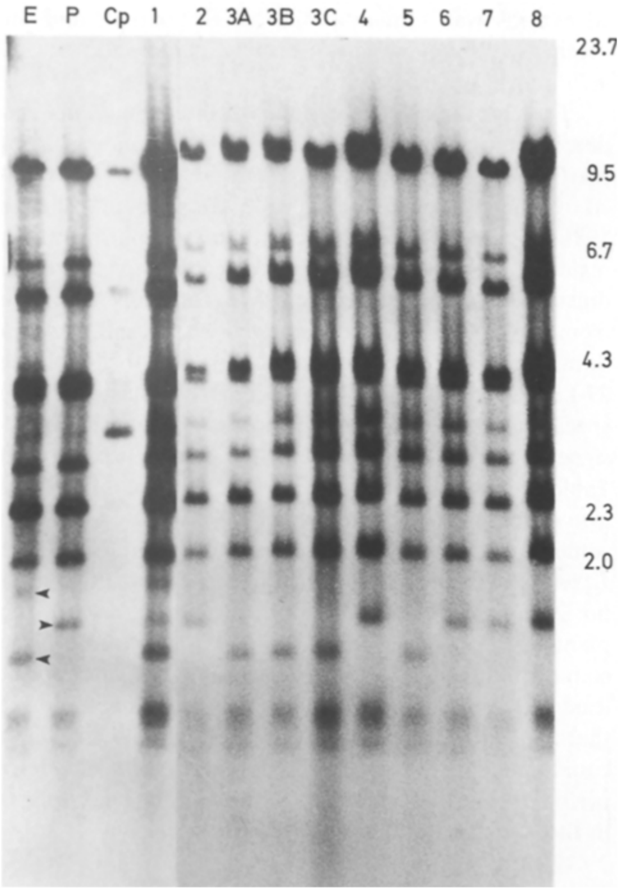

Fig. 2. Identification of mitochondrial sequences homologous to cosmid clone A2 in tomato somatic hybrids. Total DNA was isolated from leaf tissue of UC82 (E), *L. pennellii* (P), and the somatic hybrids 1–8, and purified cpDNA isolated from tomato (Cp) was also analyzed. The DNA was restricted with BamH1 and a Southern blot was prepared following electrophoresis. The blot was probed with oligolabelled cosmid clone A2. The position of lambda DNA size markers is indicated in kilobases, the position of species-specific fragments is indicated with *arrowheads*

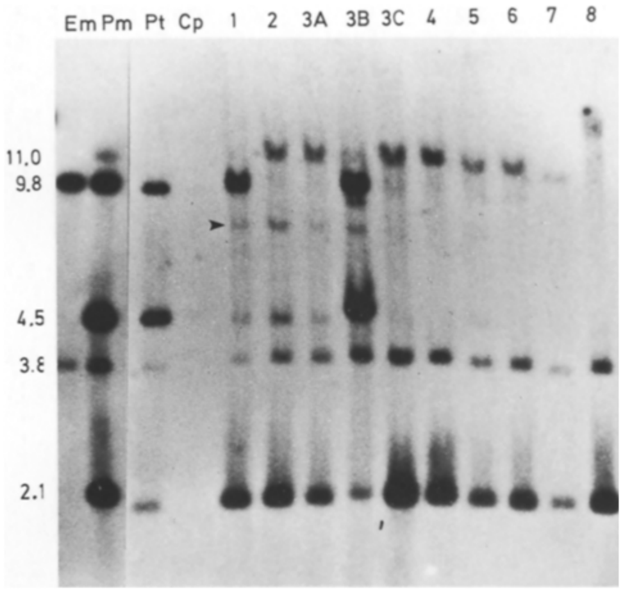

Fig. 3. Identification of mitochondrial sequences homologous to plasmid clone 2D4 in tomato somatic hybrids. Total DNA was isolated from leaf tissue of *L. pennellii* (P) and the somatic hybrids 1–8, purified cpDNA was isolated from tomato (Cp), and purified mtDNA was isolated from leaf tissue of tomato (Em) and from suspension cells of *L. pennellii* (Pm). The DNA was restricted with Sal1 and a Southern blot was prepared following electrophoresis. The blot was probed with oligolabelled plasmid clone 2D4. The position of the Sal fragments in *L. pennellii* mtDNA which hybridize to 2D4 are indicated in kilobases. The position of novel fragments in the somatic hybrids is indicated with an *arrowhead*

be identified – 11, 4.5, and 2.1 kbp (McClean and Hanson 1986; Fig. 3). There are no fragments uniquely present in the mtDNA of tomato. We have hypothesized that the sequences on the 2.1-kb Sal fragment clone designated 2D4 are repeated sequences, since they hybridize to a number of Sal fragments. In addition, the stoichiometry of the fragments that hybridize to clone 2D4 vary with the source of the DNA. When total DNA isolated from leaf tissue of *L. pennellii* is used for the Sal digestion, 2D4 hybridizes strongly to the 4.5- and 9.8-kb fragments and the signal from the 11-kb fragment is very weak (Fig. 3). The source of the mtDNA in Fig. 3, lane Pm, and for the clone 2D4 was suspension-cultured cells of *L. pennellii.* The variation in the stoichiometry of the fragments, which hybridize to 2D4 in DNA isolated from leaf versus cell suspensions, may reflect varying abundances of the origanizations of the mtDNA that give rise to these Sal fragments.

The organization of the DNA sequences homologous to 2D4 in the somatic hybrids is different from either parental organization. All of the somatic hybrids have a 3.8-kb Sal fragment present in the mtDNA of both parents, and all of the somatic hybrids have the 2.1-kb *L. pennellii*-specific Sal fragment. The presence or absence of the other Sal fragments varies with the individual somatic hybrid. For example, the hybridization patterns of somatic hybrid 3A, 3B, and 3C are quite different. Two of the three regenerants have a novel nonparental band indicated in Fig. 3 with an arrowhead. The relative abundance of the different Sal fragments also varies between the three regenerants, 3A-C. For example, the most abundant fragment in sample 3C is the 2.1-kb fragment, while in 3B the most abundant sequence is the 4.5-kb fragment. The rearrangments in the mtDNA of the somatic hybrids identified by probe 2D4 may reflect its possible function as a site of recombination in the *L. pennellii* mitochondrial genome.

Table 3. Comparison of the organization of mtDNA in tomato cybrids and somatic hybrids. Cosmid clones A1, A2, A3, B1, B3, C3, and D9, as well as the plasmid clones 2D4 and 18S + 5S genes, were used as probes. The endonucleases used to identify the RFLPs are indicated as H3, HindIII. The species-specific patterns and the percent of mtDNA RFLPs are indentified as *L. pennellii*-specific (P and % P), tomato-specific (E and % E), and of novel RFLPs (R and % R)

	2D4	A1	A2	A3	B1	B3	B3	C3	C3	D9	D9	18S	mtDNA		
Cybrids	Sal	H3	Bam	H3	Sma	Bam	H3	Sal	H3	H3	Sal	H3	% E	% P	% R
81	P+R	P	P	P	P	P	P	P	P	P	P	P	0	92	8
92A	E	E	E	E+R	E	E+R	R	E	E+R	E	E	P	66	7	27
92B	E	E	E	E+R	E	E+R	R	E+R	E+R	E	E	P	63	6	31
100A	P+R	P	E	P	E	P+E	P+E	P	P	–	P	P	29	64	7
100B	P+R	P	E	P	E	P+E	P+E	P	P	P	P	P	26	67	7
100C	P+R	P	P	P	P	P	P+E	P	P	P	P	P	7	86	7
100D	P+R	P	E	P	E	P+E	P+E	P	P	P	P	P	26	67	7
100E	P+R	P	E	P	E	P+E	P+E	P	P	–	P	P	29	64	7
121	P+R	P	P	P+R	P	P	P	P	P	P	P	P	0	86	14
122	P+R	P	P	P	P	P	P	P	P	–	P	P	0	92	8
Somatic hybrids															
1	P+R	P	E+P	E	E	P+E	E	E	E	P+E	E	–	60	30	10
2	P+R	P	P	P	P	P	P	P	P	P	P	P	0	92	8
3A	P+R	P	E	P	E	P	P	P	P	P	P	E	23	69	8
3B	P+R	P	E	P+R	–	P	P	P	P	P	P	P	8	77	15
3C	P+R	P	E	P+R	E	P	P	P	P	P	P	P	15	70	15
4	P+R	P	P	P	P	P	P	P	P	P	P	–	0	92	8
5	P+R	P	E	P	–	P	P	P	P	P	P	–	9	82	9
6	P+R	P	P	P	P	P	P	P	P	P	P	P	0	92	8
7	P+R	P	P	P	–	P	P	P	P	P	P	–	0	91	9
8	P+R	P	P	P+R	P	P	P+E	P	P	P	P	–	7	79	14

Comparison of the mtDNA organization in tomato somatic hybrids and cybrids

In an attempt to determine if the organization of the mtDNA in tomato fusion products is influenced by the nuclear genotype, a direct comparison of the mtDNA in tomato somatic hybrids and cybrids was performed. The organization of the mitochondrial genome in the tomato cybrids has already been described (Bonnema et al. 1990), and a summary of the results of that analysis has been reproduced in Table 3. Altogether, seven cosmids and two plasmid clones were used to characterize the organization of the mtDNA in the somatic hybrids and cybrids. Using the same combinations of probes and restriction endonuclease digestions, the relative abundances of tomato-specific and *L. pennellii*-specific organizations of mtDNA in the regenerants can be gauged. The relative frequencies of tomato-specific RFLPs (%E), *L. pennellii*-specific RFLPs (%P), or novel nonparental patterns (%R) were calculated from the E, P, or R scores listed in Table 3.

With the exception of two cybrids, 92A and 92B, more than 50% of the species-specific RFLPs detected were from *L. pennellii*. Most of the species-specific mtDNA sequences in the fusion products, somatic hybrids and cybrids, were *L. pennellii*-specific (63–77% on average). There is probably more mtDNA/cell in the suspension cells than in the leaf mesophyll cells used in the initial fusion construction for the production of both the somatic hybrids and the cybrids (Hanson et al. 1986). Consequently, initially there was probably more *L. pennellii* mtDNA per fused cell. This might account for the higher amount of *L. pennellii*-specific mtDNA sequences in both types of fusion products.

In general, the cybrids had more tomato-specific mtDNA sequences than did the somatic hybrids. On average, 25% of the species-specific mtDNA RFLPs in the cybrids were tomato-specific versus 12% in the somatic hybrids (Table 3). One explanation for the difference in the amount of tomato-specific mtDNA in the cybrids versus the somatic hybrids is the influence of the nuclear genome. The persistence of more *L. pennellii* mtDNA in the somatic hybrids could be a reflection of the presence of *L. pennellii* nuclear genes in the somatic hybrids.

The ten somatic hybrid plants, selected for the analysis of the organellar genomes, were chosen at random from a larger population of somatic hybrids. The ten cybrid plants selected for comparison, were identified from a larger population of regenerants that displayed only *L. esculentum* molecular markers in nuclear, chloroplast, and mitochondrial genomes (Bonnema et al. 1990). Many, if not most, of the regenerants in the cybrid experiments, which scored as *L. esculentum,* were also fusion products, but they had lost all of the *L. pennellii* cpDNA and apparently lost most, if not all, of the *L. pennellii* mtDNA. The recovery of such a large number of regenerants with no detectable *L. pennellii* organellar DNA further supports the hypothesis that nuclear genes may contribute to the inheritance of organellar genomes in an initially mixed cytoplasm.

Novel nonparental fragments were found in the ten cybrids and ten somatic hybrids analyzed. On average, the somatic hybrids and cybrids had the same frequency of novel fragments, 10–12%. Two cybrids, 92A and 92B, had a much higher frequency of novel fragments: 30%. All of the fusion products, with the exception of 92A and 92B, had nonparental hybridization patterns with the probe 2D4, a putative repeated sequence in *L. pennellii* mtDNA.

While novel fragments have been reported in somatic hybrids and cybrids, the percent of the mitochondrial genome organized in nonparental forms has not frequently been derived. Using the published descriptions of the mtDNA in several examples of cybrids and somatic hybrids (Jourdan et al. 1989; Morgan and Maliga 1987; Boeshore et al. 1983), the frequency of novel fragments in the tomato cybrids appears to be higher than reported in other systems. One explanation for a higher frequency of novel fragments could be the divergence of the parental mtDNAs. McClean and Hanson (1986) reported the mtDNA divergence between *L. esculentum* and *L. pennellii* to be 18.5 bp differences per kilobase pair. Palmer and Hebron (1988) reported that the *Brassica* mtDNAs have evolved at a much slower rate, 4 bp per kilobase pair. If novel fragments arise through intergenomic homologous recombination, then fusions of species with similar mitochondrial genome organizations should result in a low frequency of novel fragments in the mtDNA of the fusion products. In this case there could be a high frequency of intergenomic recombination, but most of these events would be silent, using RFLP analysis to detect the recombination. Using these assumptions, then one would predict that the frequency of novel parental RFLPs should be higher in fusion combinations where the parental mtDNAs differed in organization and sequence, but were similar enough to support intergenomic homologous recombination.

Acknowledgements. The author would like to thank Elizabeth Estabrook, Maureen Hanson, S. Levings, P. McClean, Madge Rothenberg, and Ellora Young for providing clones of mitochondrial genes and sequences. This reseach was supported by USDA Competitive Grant 87-CRCR-1-2291, the New Mexico Agricultural Experiment Station, and the Rio Grande Research Corridor.

References

Belliard G, Vedel F, Pelletier G (1979) Mitchondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion. Nature 218:401–403

Boeshore ML, Lifshitz I, Hanson MR, Izhar S (1983) Novel composition of mitochondrial genomes in *Petunia* somatic hybrids derived from cytoplasmic male sterile and fertile plants. Mol Gen Genet 190:459–467

Bonnema AB, Melzer JM, O'Connell MA (1991) Tomato cybrids with mitochondrial DNA from *Lycopersicon pennellii.* Theor Appl Genet 81:339–348

Braun CJ, Levings CS (1985) Nucleotide sequence of the F_1-ATPase α-subunit gene from maize mitochondria. Plant Physiol 79:571–577

Brears T, Curtis GJ, Lonsdale DM (1989) A specific rearrangement of mitochondrial DNA induced by tissue culture. Theor Appl Genet 77:620–624

Chao S, Sederoff R, Levings CS (1984) Nucleotide sequence and evolution of the 18S ribosomal RNA gene in maize mitchondria. Nucleic Acids Res 12:6629–6644

Chetrit P, Mathieu C, Vedel F, Pelletier G, Primard C (1985) Mitochondrial DNA polymorphism induced by protoplast fusion in Cruciferae. Theor Appl Genet 69:361–366

Dewey RE, Levings CS, Timothy DH (1985) Nucleotide sequence of ATPase subunit 6 gene of maize mitochondria. Plant Physiol 79:914–919

Dudits D, Hadlaczky Gy, Lazar G, Haydu Z (1980) Increase in genetic variability through somatic cell hybridization of distantly related plant species. In: Sala F, Rarisi B, Cella R, Ciferri O (eds) Plant cell cultures: results and perspectives. Elsevier, Amsterdam, pp 207–214

Estabrook EM, O'Connell MA (1986) Organization of the 18S+5S ribosomal RNA gene in tomato mitochondrial DNA, Int Workshop Higher Plant Michondrial DNA, Airlie/VA (abstract)

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Fox TD, Leaver CJ (1981) The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. Cell 26:315–323

Galun E, Aviv D (1983) Cytoplasmic hybridization: genetics and breeding applications. In: Evans DA, Sharp WR, Ammirato PV, Yamada Y (eds) Handbook of plant cell culture, vol 1. MacMillan, New York, pp 358–392

Hanson MR, Boeshore MB, McClean PE, Nivison HT, O'Connell MA (1986) The isolation of mitochondria and mitochondrial DNA. Methods Enzymol 118:437–445

Hartmann C, Henry Y, De Buyser J, Aubry C, Rode A (1989) Identification from new mitochondrial genome organizations in wheat plants regenerated from somatic tissue cultures. Theor Appl Genet 77:169–175

Hause B, Baldauf F, Stock K, Wasternack C, Metzlaff M (1986) Molecular analysis of mitochondrial DNA from tomato cell suspension cultures. Curr Genet 10:785–790

Hohn B, Collins J (1980) A small cosmid for efficient cloning of large DNA fragments. Gene 11:291–298

Jourdan PS, Earle ED, Mutschler MA (1989) Synthesis of male sterile, triazine-resistant *Brassica napus* by somatic hybridization between cytoplasmic male sterile *B. oleracea* and atrazine-resistant *B. campestris.* Theor Appl Genet 78:445–455

Kemble RJ, Barsby TL, Yarrow SA (1988) Transformation of plant mitochondria with mitochondrial DNA plasmids via protoplast fusion. Mol Gen Genet 213: 202–205

Laughnan JR, Gabay-Laughnan S (1983) Cytoplasmic male sterility in maize. Annu Rev Genet 17: 27–48

Lonsdale DM, Hodge TP, Fauron CMR, Flavell RB (1983) A predicted structure for the mitochondrial genome from the fertile cytoplasm of maize. In: Goldberg RB (ed) Plant molecular biology, vol 12 New Series. Alan R. Liss, New York, pp 445–456

McClean PE, Hanson MR (1986) Mitochondrial DNA sequence divergence among *Lycopersicon* and related *Solanum* species. Genetics 112: 649–667

McNay JW, Choury PS, Pring DR (1984) Molecular analysis of genomic stability of mitochondrial DNA in tissue-cultured cells of maize. Theor Appl Genet 67: 433–437

Medgyesy P, Fejes E, Maliga P (1985) Interspecific chloroplast recombination in a *Nicotiana* somatic hybrid. Proc Natl Acad Sci USA 82: 6960–6964

Morgan A, Maliga P (1987) Rapid chloroplast segregation and recombination of mitochondrial DNA in *Brassica* cybrids. Mol Gen Genet 209: 240–246

O'Connell MA, Hanson MR (1987) Regeneration of somatic hybrid plants formed between *Lycopersicon esculentum* and *Lycopersicon pennellii*. Theor Appl Genet 75: 83–89

Palmer JD Hebron LA (1988) Plant mitochondrial DNA evolves rapidly in structure, but slowly in sequence. J Mol Evol 28: 87–97

Palmer JD, Shields CR (1984) Tripartite structure of the *Brassica campestris* mitochondrial genome. Nature 307: 437–439

Palmer JF, Zamir D (1982) Chloroplast DNA evolution and phylogenetic relationships in *Lycopersion*. Proc Natl Acad Sci USA 78: 5533–5536

Phillips AL (1985) Restriction map and clone bank of tomato plastid DNA. Curr Genet 10: 147–152

Rothenberg M, Boeshore ML, Hanson MR, Izhar S (1985) Intergenomic recombination of mitochondrial genomes in a somatic hybrid plant. Curr Genet 9: 615–618

Saltz Y, Beckmann J (1981) Chloroplast DNA preparation from *Petunia* and *Nicotiana*. Plant Mol Biol Newslett 2: 73

Shepard JF, Bidney D, Barsby T, Kemble R (1983) Genetic transfer in plants through interspecific protoplast fusion. Science 219: 683–688

Shirzadegan M, Christey M, Earle ED, Palmer JD (1989) Rearrangement, amplification, and assortment of mitochondrial DNA molecules in cultured cells of *Brassica campestris*. Theor Appl Genet 77: 17–25

Small ID, Isaac PG, Leaver CJ (1987) Stoichiometric differences in DNA molecules containing atpA gene suggest mechanisms for the generation of mitochondrial genome diversity in maize. EMBO J 6: 865–869

Stern DB, Dyer TA, Lonsdale DM (1982) Organization of the mitochondrial ribosomal RNA genes of maize. Nucleic Acids Res 10: 3333–3340

Thanh ND, Medgyesy P (1989) Limited chloroplast gene transfer via recombination overcomes plastome-genome incompatibility between *Nicotiana tabacum* and *Solanum tuberosum*. Plant Mol Biol 12: 87–93

Ward BL, Anderson RS, Bendich AJ (1981) The mitochondrial genome is large and variable in a family of plants (Cucurbitaceae). Cell 25: 793–803

Young EG, Hanson MR, Dierks PM (1986) Sequence and transcription of the petunia gene for the ATP synthase proteolipid subunit. Nucleic Acids Res 14: 7995–8006