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Differential fate of plastid and mitochondrial genomes in *Petunia* somatic hybrids

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Summary. The chloroplast (cp) and mitochondrial (mt) DNAs of *Petunia* somatic hybrid plants, which were derived from the fusion of wild-type *P. parodii* protoplasts with albino *P. inflata* protoplasts, were analyzed by endonuclease restriction and Southern blot hybridization. Using ^{32}P-labelled probes that distinguished the two parental cpDNAs at a BamHI site and at a HpaII site, only the *P. parodii* chloroplast genome was detected in the 10 somatic hybrid plants analyzed. To examine whether cytoplasmic mixing had resulted in rearrangement of the mitochondrial genome in the somatic hybrids, restriction patterns of purified somatic hybrid and parental mtDNAs were analyzed. Approximately 87% of those restriction fragments which distinguish the two parental genomes are *P. inflata*-specific. Restriction patterns of the somatic hybrid mtDNAs differ both from the parental patterns and from each other, suggesting that an interaction occurred between the parental mitochondrial genomes in the somatic fusion products which resulted in generation of the novel mtDNA patterns. Southern blot hybridization substantiates this conclusion. In addition, somatic hybrid lines derived from the same fusion product were observed to differ in mtDNA restriction pattern, reflecting a differential sorting-out of mitochondrial genomes at the time the plants were regenerated.

Key words: *Petunia* – Chloroplast DNA – Mitochondrial DNA – Somatic hybrid – Protoplast fusion

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Introduction

Somatic hybridization of plant cells provides the opportunity to combine different genetically marked cytoplasmic genomes in the same cell. Such mixtures cannot otherwise be obtained in those species which exhibit maternal-uniparental inheritance of cytoplasmic genomes in sexual hybridizations. Analysis of organelle genomes in somatic hybrid tissue and plants can reveal whether novel cytoplasmic genomes have been created through exchange of genetic material, or whether segregation of parental cytoplasmic genomes has occurred. Observations to date indicate that the chloroplast genomes of somatic hybrids assort rapidly (reviewed by Shepard et al. 1983), and may rarely undergo recombination of cpDNAs (Medgyesy et al. 1985). In contrast, much evidence exists for recombination of mitochondrial genomes in somatic hybrid plants (Belliard et al. 1979; Nagy et al. 1981; Galun et al. 1982; Boeshore et al. 1983; Rothenberg et al. 1985).

In this study the organelle genomes of somatic hybrids derived from the fusion of wild-type *Petunia parodii* and a *P. inflata* albino line were analyzed. The *P. inflata* albino mutant was determined to be the result of a nuclear-encoded mutation based on genetic and molecular analyses. The plastid genome of the albino line was not detected in the ten somatic hybrid plants tested. However, the mitochondrial genomes of these plants have undergone rearrangements that resulted in mtDNA restriction patterns containing both *P. parodii*- and *P. inflata*-specific fragments, as well as novel fragments not present in either parental genome. Moreover, while these hybrid genomes are remarkably similar in that they all contain a preponderance of *P. inflata*-specific fragments, diversity was observed among plants derived from a single fusion event.

Materials and methods

Construction of P. parodii + P. inflata somatic hybrids

P. parodii wild-type leaf mesophyll protoplasts were fused with protoplasts derived from a chlorophyll-deficient *P. inflata* line (Power et al. 1979), grown in liquid suspension culture. Selection for hybrid calli consisted of visual identification (*P. inflata* callus remained colorless while somatic hybrid callus underwent greening), and differential growth on selective medium (*P. parodii* callus did not grow beyond the 50-cell stage). Regenerated plants were confirmed as somatic hybrids based on floral and leaf morphology, anthocyanin expression, chromosome number, segregation for parental characters, and isozyme patterns (Schnabelrauch et al. 1985).

A complete description of the plant material used in this analysis is given by Schnabelrauch et al. (1985).

DNA analysis

CpDNA was isolated from leaves by the method of Salts and Beckmann (1981). Total DNA was isolated from leaves by the method of Murray and Thompson (1980). MtDNA was isolated from cell suspension as described by Boeshore et al. (1983). Electrophoresis, Southern blotting, nick-translation, hybridization, autoradiography, and photography were performed as described by Boeshore et al. (1983). Restriction enzymes were used according to the supplier's instructions.

The 2.8kb BamHI fragment of *P. inflata* cpDNA was cloned into plasmid pUC9 (Vieira and Messing 1982) according to standard procedures (Maniatis et al. 1982). The 19kb PstI fragment of *P. hybrida* cpDNA was kindly provided by J. Palmer (University of Michigan).

Results

Chloroplast genomes

Restriction enzymes BamHI, HpaII and HhaI distinguish the chloroplast genomes of *P. inflata* and *P. parodii*. Total plant DNA was prepared, restricted with BamHI or HpaII, and probed with plasmid clones which hybridized specifically to cpDNA fragments characteristic of one or the other parental line. In the ten somatic hybrid plants examined (derived from a total of five different fusion products) only the *P. parodii*-specific cpDNA fragments were detected (Fig. 1). This result precludes the possibility that a detectable recombination event had occurred with respect to the two restriction sites assayed.

The level of sensitivity of the hybridization assay was determined by reconstitution experiments in which varying amounts of *P. inflata* total DNA extracted from albino leaf tissue were mixed with total *P. parodii* DNA extracted from green leaves. The *P. inflata*-specific BamHI and HpaII cpDNA fragments were detectable in a mixture of one part *P. inflata* DNA in 200 parts *P. parodii* DNA after a 48 h exposure. Standard mixtures of 1:100 and 1:200 were included on all blots on which somatic hybrid cpDNAs were analyzed.

The *P. inflata* albino line used in this fusion experiment had been suggested to be a cytoplasmic albino based on its tissue culture origin (Power et al. 1979; Kumar et al. 1982). Because the presence of variegation in some of these somatic hybrid lines suggested the presence of cells segregating the *P. inflata* chloroplast genome, the genetic behavior of the albino phenotype was tested by germinating 675 seeds obtained by self-pollinating flowers on nonvariegated branches of line 15-6. To offset potential viability problems resulting from the inability of chlorophyll-deficient seedlings to maintain their own growth, seeds were aseptically plated on N13 medium (Hosticka and Hanson 1984). Of the 675 germinated seedlings, 5 were entirely albino. The expected percentage of plants exhibiting a homozygous recessive trait in an autotetraploid cross is approximately 3%; the fact that less than 1% of the germinated plantlets exhibited the albino trait in this cross may be a reflection of decreased viability.

To test the possibility that these albino plantlets were the product of meiotic sorting out of cytoplasmic albino plastids that had otherwise remained visually undetected in the green tissue from whence the seeds were derived, four albino seedlings were plated onto UM1a medium (Uchimiya and Murashige 1974; Hosticka and Hanson 1984). After three months, sufficient tissue had accumulated to permit extraction of total genomic DNA. Probing restricted samples of that DNA with the appropriate cpDNA probes yielded BamHI and HpaII patterns indicative of the *P. parodii* chloroplast genome (Fig. 1). These results suggest that the autotetraploid cross had segregated an albino trait encoded by the nuclear genome.

Mitochondrial genomes

The mitochondrial genomes of parental and somatic hybrid lines were examined by restriction analysis and hybridization to labelled probes. All five enzymes tested distinguished the *P. parodii* and *P. inflata* mitochondrial genomes (the restriction pattern generated by PvuII is shown in Fig. 2). A restriction band that is present in only one of the two parental genomes, and can thus be used to determine the relative representation of each parental mtDNA in a somatic hybrid mitochondrial genome, will henceforth be referred to as a "unique" band. A band that occurs in a somatic hybrid mtDNA restriction pattern but is visualized in neither parental mtDNA pattern will be referred to as a "novel" band (Boeshore et al. 1983).

A composite diagram illustrating some of the restriction pattern differences generated by five enzymes, and the pattern of distribution of the differences in the somatic hybrids, is given in Fig. 3. The electrophoretic system used in this work did not resolve all of the re-

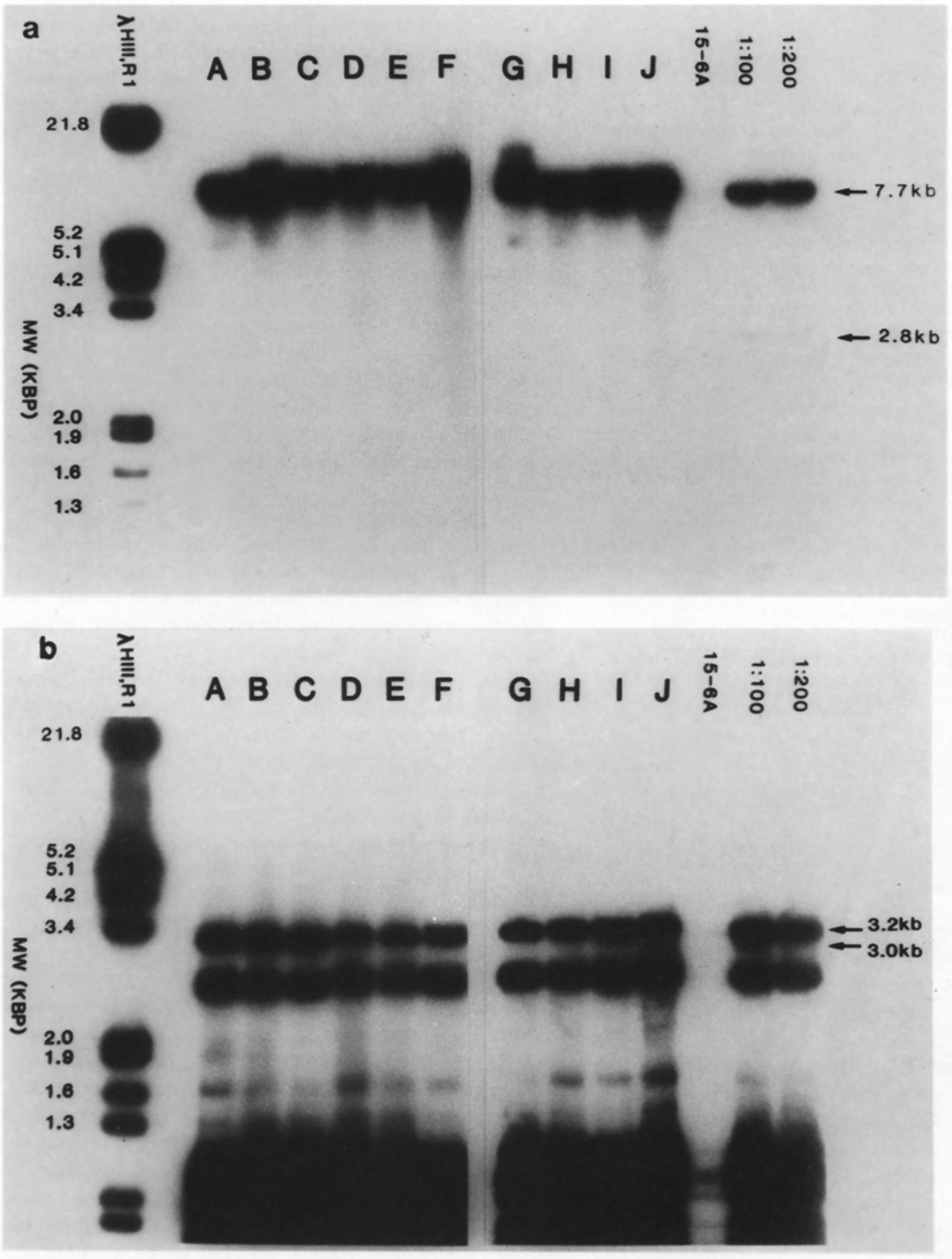

Fig. 1. **a** Autoradiogram showing hybridization of ^{32}P-labelled 2.8 kb cloned BamH1 fragment of *P. inflata* cpDNA to BamH1 digests of parental and somatic hybrid total DNA. Somatic hybrid DNAs are labelled as follows: *A*) 14-3; *B*) 20-1; *C*) 15-1; *D*) 15-2; *E*) 21-1; *F*) 21-5; *T*) 28-1; *H*) 15-3; *I*) 15-4; *J*) 15-6. "15-6A" is DNA from albino progeny of somatic hybrid line 15-6 (see text for "Description of experiment"). Lanes designated "1:100" and "1:200" contain mixtures of *P. inflata* DNA and *P. parodii* DNA in ratios of 1 to 100 and 1 to 200. Locations of the *inflata*- and *parodii*-specific restriction bands are indicated by arrows. λ HIII, R1 is lambda DNA double-digested with HindIII and EcoR1. Approximately 8 μg of DNA was loaded in wells *A* through *J*; 5 μg of DNA was loaded in lanes "1:100" and "1:200". The 2.8 kb *inflata*-specific band is visible in the 1:200 lane; thus, the level of detection for the *inflata*-specific band obtained for *lanes A* through J is less than 0.5%. Electrophoresis was performed in 1.0% agarose at 70 V for 10 h. ^{32}P-labelled lambda DNA was included in the probe. **b** Autoradiogram showing hybridization of ^{32}P-labelled 19 kb cloned Pst 1 fragment of *P. hybrida* cpDNA to HpaII digests of parental and somatic hybrid total DNA. Conditions and abbreviations are as described above. The level of detection for the 3.0 kb *inflata*-specific band is between 0.5% and 1.0%

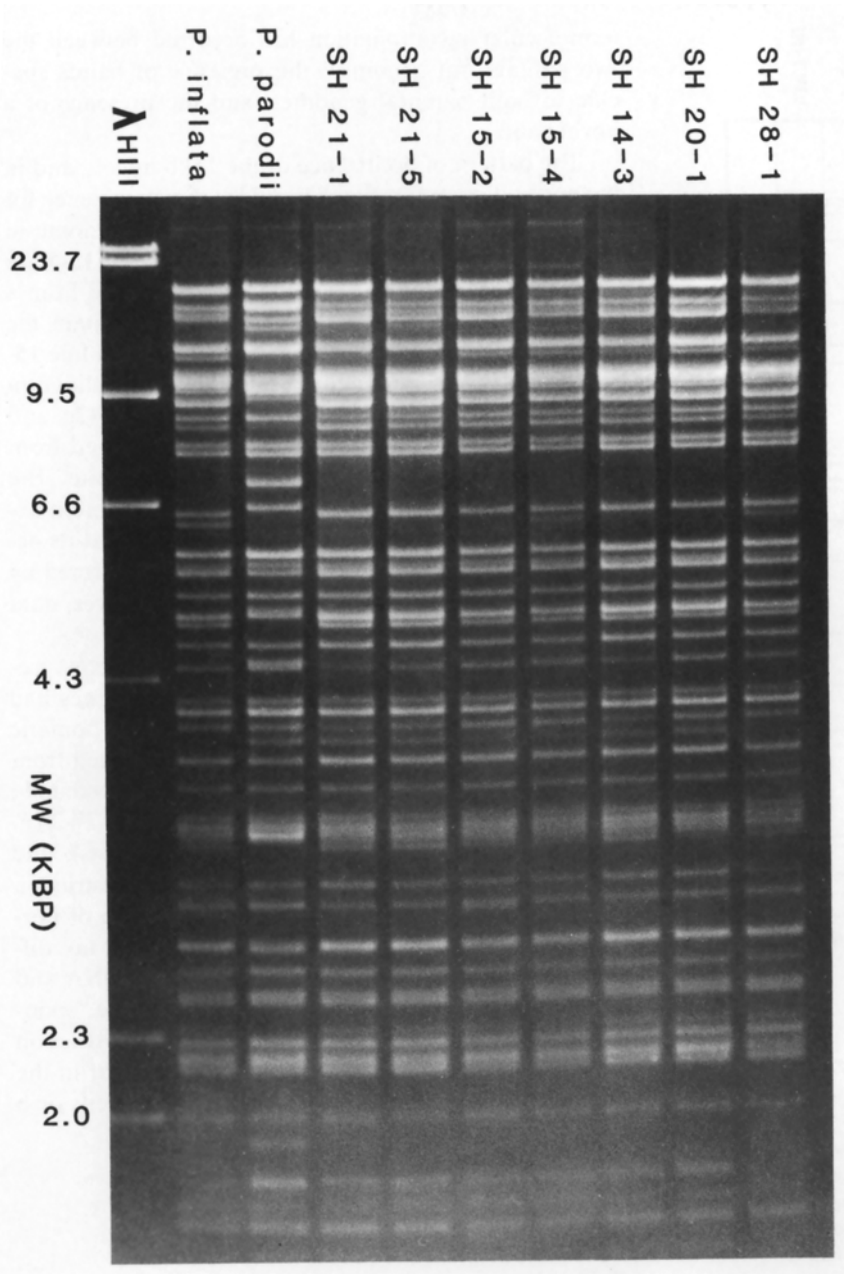

Fig. 2. Photograph of an agarose gel following electrophoresis of purified parental and somatic hybrid mtDNAs digested with PvuII. Somatic hybrid mtDNA is indicated by "SH" followed by a number identifying the somatic hybrid plant. λ HIII is lambda DNA digested with HindIII. Approximately 2 μg mtDNA was loaded in each well. Electrophoresis was performed in 1.0% agarose at 60 V for about 12 h

striction bands; many fragments of similar molecular weight, which may or may not have been similar with respect to sequence content, were observed to comigrate. Therefore, only those bands that were clearly present in one parental species and absent in the other, as visualized on ethidium-stained gels, were included in the composite diagram. A composite was generated in order to utilize all of the available data.

Analysis of the composite diagram (Fig. 3) allows several ovservations. Restriction patterns of the purified mtDNAs from all of the somatic hybrids are different from those of either parent. This observation is indicative of recombination and/or rearrangement of the parental genomes in the heterokaryons (Belliard et al. 1979; Nagy et al. 1981; Galun et al. 1982; Boeshore et al. 1983; Rothenberg et al. 1985). Though 87% of the unique bands observed in the somatic hybrids are *P. inflata*-specific, in no instance were all of the *P. inflata*-specific (or all of the *P. parodii*-specific) bands transmitted to a somatic hybrid mitochondrial genome, ruling out the possibility that a simple summation of the two genomes had occurred, or that one of the parental genomes had been transmitted in its entirety, without rearrangement.

Hybridization analysis confirms and extends these conclusions. The autoradiogram in Fig. 4 illustrates several observations. The somatic hybrids all contain one band that is specific to *P. inflata*. They also contain two

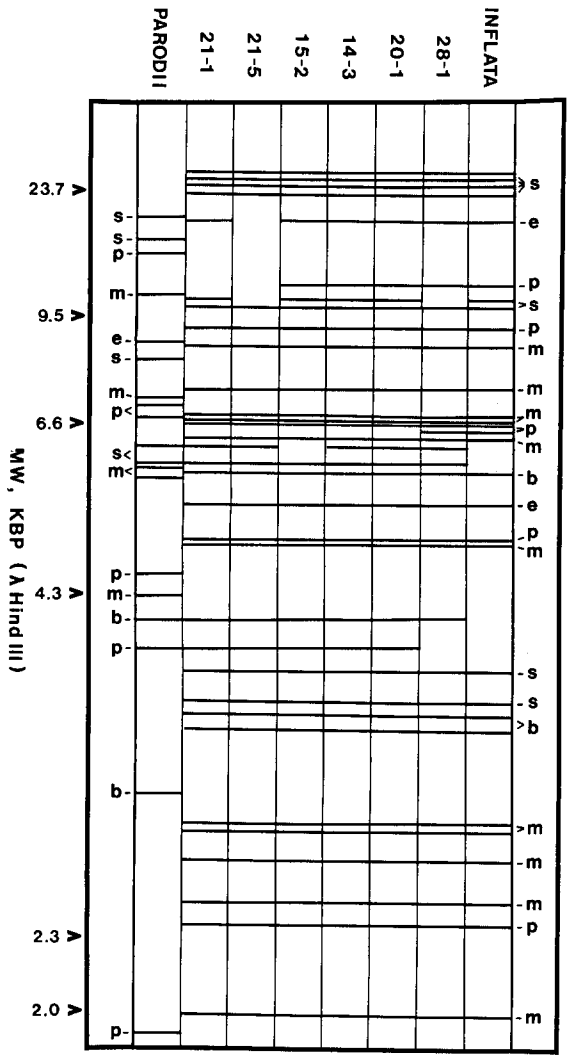

Fig. 3. Diagram showing a composite of restriction pattern differences between *P. inflata, P. parodii,* and somatic hybrid mtDNAs. Lanes labelled with numbers represent individual somatic hybrid lines. This composite represents the sum of differences detected using five enzymes (see text). Individual bands representing pattern differences are labelled as follows: *s* (Sst 1), *e* (EcoR 1), *p* (Pvull), *m* (Sma 1), and *b* (Bgl 1)

P. parodii-specific bands, although in varying stoichiometries. One band, common to both parents, is present in all somatic hybrids; a single *P. parodii*-specific band is missing in all somatic hybrids.

The visualization of "novel" bands in restriction patterns of somatic hybrid mtDNAs has been presented as evidence for intermolecular recombination between the two parental mt genomes in the generation of the somatic hybrid mt genome (Belliard et al. 1979). A 15kb novel band is present in the somatic hybrid genomes represented by SH15-2g and SH15-2v in the autoradiogram in Fig. 4. Thus two indications exist (as represented in this autoradiogram) suggesting that intermolecular recombination has occurred between the two parental mt genomes: the presence of bands specific to both parental genomes, and the presence of a novel band.

The pattern of occurrence of the 15kb novel band in the autoradiogram in Fig. 4 provides direct evidence for differential sorting-out of the mitochondrial genome in regenerating plantlets. Lines 15-2g, 15-2v, and 15-4 are all derived from callus tissue that was produced from a single fusion event. Lines 15-2g and 15-2v contain the 15kb novel band. This band was not detected in line 15-4. The mtDNAs represented on this autoradiogram were purified from suspension cells; lines 15-2g and 15-2v were two different suspension lines derived from different cuttings of the original 15-2 regenerate. The fact that the novel band was present in two different explants from one of the regenerates indicates that its occurrence was not a tissue culture artefact (confirmed by hybridization to total DNA extracted from leaves, data not shown).

To examine the possibility that spontaneous rearrangements in the mtDNA of cultured cell lines had contributed to the variability observed in the somatic hybrid restriction patterns, mtDNA was extracted from lines 80-11-2 (the 2n sexual hybrid; *P. parodii* as female parent crossed with *P. inflata* as male parent), 77-2 (*P. parodii* regenerated from mesophyll protoplasts), and 77-22-4x-3 (a tetraploid *P. parodii* line). The restriction patterns of these lines were compared to those of normal diploid *P. parodii* with three enzymes; no differences were detected between *P. parodii* mtDNA and any of these lines (data not shown). Therefore, spontaneous changes did not occur in the mtDNA restriction patterns during regeneration from protoplasts, or in the presence of the sexually produced hybrid nucleus or a tetraploid *P. parodii* nucleus.

Discussion

Chloroplast genomes

Kumar et al. (1982) analyzed purified cpDNA from three different somatic hybrids (*P. parodii* + *P. hybrida, P. parodii* + *P. inflata,* and *P. parodii* + *P. parviflora*) using the enzymes HpaII and BamHI. Each somatic hybrid line tested was derived from several plants propagated from a single fusion product, and only the *P. parodii* chloroplast genome was detected in each of these somatic hybrids. Visual detection of a band representing a minority cpDNA population in a mixture of two restricted chloroplast DNAs on ethidium-stained gels permits resolution to within about 5% in cases where the species-specific bands do not comigrate with other bands (Scowcroft and Larkin 1981).

The analysis presented here parallels the observations of Kumar et al. (1982) on their *P. parodii* + *P. inflata* somatic hybrid, while extending the limit of detection to 0.5%.

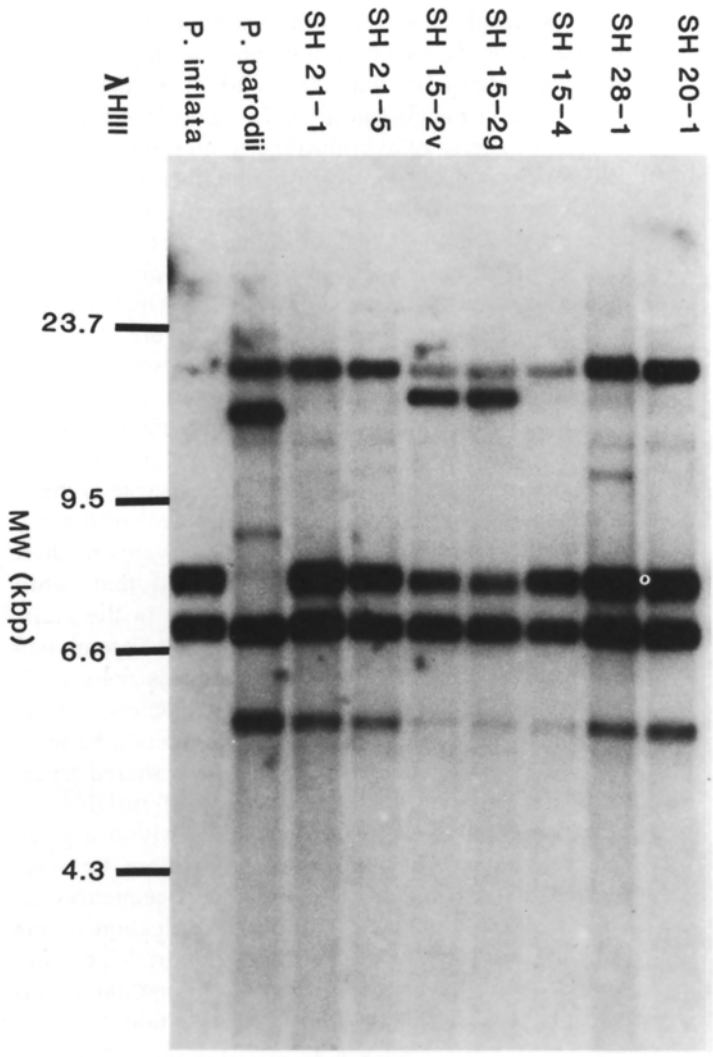

Fig. 4. Autoradiogram showing hybridization of a cloned 9.5 kb BamH1 fragment of *P. hybrida* mtDNA (plasmid p 133EC11; Boeshore et al. 1983) to BstEII digests of parental and somatic hybrid mtDNAs. Abbreviations are as described in Fig. 1. Approximately 2 μg mtDNA was loaded in each well. Electrophoresis was performed in 0.9% agarose at 70 V for about 20 h

As discussed by Schnabelrauch et al. (1985), some of the somatic hybrids used in this study exhibited such morphological aberrations as corolla and leaf pigment variegation, floral dimension changes, and reduced pollen viability. The phenotypic changes were postulated to be a result of cytological instability in a biparental cytoplasm, nuclear-cytoplasmic genomic incompatibility, or a phenomenon similar to hybrid dysgenesis occurring as a result of somatic fusion. Leaf variegation was observed in 27% of *P. parodii* + *P. inflata* somatic hybrid regenerates. The results presented here rule out the possibility that the variegation is due to the presence of chlorophyll-deficient *P. inflata* plastids. The *P. inflata* albino parental line had been previously suggested to harbor a cytoplasmically-inherited chlorophyll deficiency (Power et al. 1979; Kumar et al. 1982); in contrast, the results presented here indicate that the chlorophyll deficiency is nuclear-encoded. First, the *P. inflata* chloroplast genome was detected neither in green nor in variegated somatic hybrid plants. Second, progeny obtained from a somatic hybrid by self-pollinating flowers on nonvariegated branches segregated for chlorophyll deficiency; the albino progeny exhibited the cpDNA restriction pattern of *P. parodii.* The latter result suggests that the *P. inflata* albino parent harbored a recessive nuclear mutation that was expressed in the somatic hybrid progeny only in the homozygous condition. In addition, *P. inflata* albino plants maintained in culture over a period of four years occasionally exhibited small, well-defined green spots (E. Clark, unpublished results). The observed pattern was indicative of reversion of a nuclear mutation rather than vegetative segregation of a chloroplast genome that had reverted to a green phenotype; the complex multiple-cell-lineage chequered pattern characteristic of chloroplast sorting-out (Kirk and Tilney-Bassett 1978) was never observed.

In several published cases, there existed an apparent bias in favor of one parental chloroplast type in somatic hybrid re-

generates, evidenced by the exclusive presence of one chloroplast genome in all regenerates (Evans et al. 1980; Maliga et al. 1980; Bonnett and Glimelius 1983; Clark et al. 1985). Scowcroft and Larkin (1981) discussed the factors which might influence the outcome of chloroplast segregation, including nucleocytoplasmic incompatibilities between species, physiological differences in the states of parental protoplasts, and selection pressures intrinsic to the fusion protocol.

Several factors may have contributed to the exclusive presence of *P. parodii* cpDNA in the somatic hybrids analyzed in this study. The selection system utilized as part of the fusion protocol involves selecting green calli from a white background, which automatically excludes tissue that does not contain either a preponderance of *P. parodii* chloroplasts or *P. inflata* chloroplasts that exhibit a restored photoautotrophy. It is possible that the *P. parodii* nuclear genome present in the tetraploid somatic hybrid nucleus was not capable of restoring photoautotrophy to the *P. inflata* plastids. This question cannot be tested by sexual crosses because the two species are unilaterally cross-incompatible; sexual hybrids can only be generated using *P. parodii* as the female parent (Sink et al. 1978). It is unlikely that nuclear-cytoplasmic incompatibility per se is a factor in the cross-incompatibility, however, since the two species exhibit a pre-zygotic mode of reproductive isolation.

The physiological state of protoplasts used in the fusion protocol may also have influenced the chloroplast composition of the fusion products; *P. parodii* protoplasts were derived from leaf mesophyll, while *P. inflata* protoplasts were derived from suspension cells. It is possible that a difference in the number and/or reproduction rate of plastids in the two cell types influenced the sorting-out process (Clark et al. 1985).

Mitochondrial genomes

Belliard et al. (1979) showed that the mtDNA restriction patterns of nine *Nicotiana* somatic hybrids differed from those of the parental genomes, providing the first evidence for recombination of plant mitochondrial genomes. Galun et al. (1982) obtained similar results in an analysis of the mtDNA restriction patterns of seven *Nicotiana* somatic hybrids. Nagy et al. (1981) used a plasmid containing *E. coli* rDNA as a probe in hybridizations to restricted parental and somatic hybrid mtDNAs, and obtained hybridization to novel bands present only in the somatic hybrid mtDNAs. Using cloned mtDNA fragments, Boeshore et al. (1983) showed that a set of *Petunia* somatic hybrid mtDNAs consisted of novel combinations of restriction fragments derived from both parents. Rothenberg et al. (1985) later provided direct evidence that a mtDNA region in one of these *Petunia* somatic hybrids was derived from intermolecular recombination.

The results in the analysis presented here differ from previous observations in two respects. First, within the eight lines examined, there was little heterogeneity in restriction pattern; two lines were indistinguishable (somatic hybrids 14-3 and 20-1; diagrammed in Fig. 3). Of the 34 unique bands detected in the somatic hybrids using 5 enzymes (Fig. 3), 28 were present in all somatic hybrids tested. However, some variation was observed between lines derived from the same fusion product; restriction patterns of lines 21-1 and 21-5 differed (diagrammed in Fig. 3), and lines 15-2 and 15-4 exhibited different patterns of hybridization to plasmid p133EC11 (Fig. 4). Second, a majority of parental-specific mtDNA bands were derived from *P. inflata,* in contrast to the chloroplast genome, which exhibited only the *P. parodii* restriction pattern. A *P. parodii* × *P. inflata* hybrid nucleus can support a *P.parodii* cytoplasm, which suggests that any selection pressure in favor of the *P. inflata* mitochondrial genome is not a result of nucleocytoplasmic incompatibility.

Consideration of the models of organization of the *Brassica* (Palmer and Shields 1984) and maize (Lonsdale et al. 1984) mitochondrial genomes suggests that a site-specific recombination system may govern the majority of intramolecular recombination events in these systems. Lonsdale et al. (1984) observed that, while there are other repeat sequences present in the maize mt genome, intramolecular recombination appears limited to certain repeats. If recombination is site-specific and is thus limited to certain repeat sequences, then in order to achieve intermolecular recombination between two different mt genomes there must be a shared repeat sequence between them. The degree of mtDNA restriction pattern heterogeneity possible within a given set of somatic hybrid plants may therefore be a reflection of the number of shared repeat sequences involved in recombination. Further investigation of the structure of somatic hybrid mitochondrial genomes should define the nature of the recombination events which have generated their novel organization.

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References

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

Boeshore ML, Hansol MR, Lifshitz I, 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

Bonnett HT, Glimelius K (1983) Somatic hybridization in *Nicotiana:* behavior of organelles after fusion of protoplasts from male-fertile and male-sterile cultivars. Theor Appl Genet 65:213–217

Clark EM, Izhar S, Hanson M (1985) Independent segregation of the plastid genome and cytoplasmic male sterility in *Petunia* somatic hybrids. Mol Gen Genet 199:440–445

Evans DA, Wetter LR, Gamborg OL (1980) Somatic hybrid plants of *Nicotiana glauca* and *N. tabacum* obtained by protoplast fusion. Physiol Plant 48:225–230

Galun E, Arzee-Gonen P, Fluhr R, Edelman M, Aviv D (1982) Cytoplasmic hybridization in *Nicotiana:* Mitochondrial DNA analysis in progenies resulting from fusion between protoplasts having different organelle DNA constitutions. Mol Gen Genet 186:50–56

Hosticka LP, Hanson MR (1984) Induction of plastid mutations in tomatoes by nitrosomethylurea. J Hered 75:242–246

Kirk JTO, Tilney-Bassett RAE (1978) The plastids. Elsevier/ North Holland Biomedical Press, New York, Amsterdam

Kumar A, Cocking EC, Bovenberg WA, Kool AJ (1982) Restriction endonuclease analysis of chloroplast DNA in interspecies somatic hybrids of *Petunia.* Theor Appl Genet 62:377–383

Lonsdale DM, Hodge TP, Fauron CM-R (1984) The physical map and organization of the mitochondrial genome from the fertile cytoplasm of maize. Nucleic Acids Res 12:9249–9261

Maliga P, Nagy F, Xuan LT, Kiss ZsR, Menczel L, Lazar G (1980) Protoplast fusion to study cytoplasmic traits in *Nicotiana.* In: Advances in protoplast research. Academiai Kiado, Budapest (Hungary), pp 341–345

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning. Cold Spring Harbor Laboratories, Cold Spring Harbor NY

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

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nagy F, Torok I, Maliga P (1981) Extensive rearrangements in the mitochondrial DNA in somatic hybrids of *Nicotiana tabacum* and *Nicotiana knightiana.* Mol Gen Genet 183:437–439

Palmer JD, Shields CR (1984) Tripartite structure of the *Brassica campestris* mitochondrial genome. Nature 307:437–440

Power JB, Berry SF, Chapman JV, Cocking EC (1979) Somatic hybrids between unilateral cross-incompatible *Petunia* species. Theor Appl Genet 55:97–99

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

Salts Y, Beckman J (1981) Chloroplast DNA preparation from *Petunia* and *Nicotiana.* Plant Mol Biol Newslett 2:73–74

Schnabelrauch LS, Kloc-Bauchan F, Sink KC (1985) Expression of nuclear-cytoplasmic genomic incompatibility in interspecific *Petunia* somatic hybrid plants. Theor Appl Genet 70:57–65

Scowcroft WR, Larkin PJ (1981) Chloroplast DNA assorts randomly in intraspecific somatic hybrids of *Nicotiana debneyi.* Theor Appl Genet 60:179–184

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

Sink KC, Power JB, Natarella NJ (1978) The interspecific hybrid *Petunia parodii* × *P. inflata* and its relevance to somatic hybridization in the genus *Petunia.* Theor Appl Genet 53:205–208

Uchimiya H, Murashige T (1974) Evolution of parameters in the isolation of viable protoplasts from cultured tobacco cells. Plant Physiol 54:936–944

Vieira J, Messing J (1982) The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19:259–268