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Analysis of chloroplast and mitochondrial segregation in three different combinations of somatic hybrids produced within *Brassicaceae*

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Summary. Mitochondrial and chloroplast DNA were characterized in three different combinations of somatic hybrids produced between different species within *Brassicaceae*. The fusions were made between *B. campestris* and *B. oleracea*, *B. napus* and *B. nigra* and between *B. napus* and *Eruca sativa*. The combinations represent interspecific hybridizations, but the phylogenetic distance between the species used in each instance is different. Whereas the *B. campestris* (+) *B. oleracea* and the *B. napus* (+) *B. nigra* hybrids are both examples of intrageneric hybrids, *B. campestris* is more closely related to *B. oleracea* than *B. napus* is to *B. nigra*. The fusion of *B. napus* and *E. sativa* represents an intergeneric hybridization. Since hybrids were produced with reproducible and uniform fusion and culture methods, a comparison of chloroplast and mitochondrial segregation and mitochondrial DNA (mt-DNA) rearrangements could be made between the combinations. The segregation of both chloroplasts and mitochondria was biased in the *B. napus* (+) *B. nigra* and the *B. napus* (+) *E. sativa* combination. The nonrandom segregation of chloroplasts and mitochondria could be due to the different ploidy levels of the fusion partners and/or reflect differences in organelle replication rate. Furthermore, segregation of mitochondria was correlated to the differences in phylogenetic distance between the species used in the fusions. However, mitochondrial segregation, in contrast to chloroplast segregation, could in all combinations also have been affected by the cell type used as protoplast source in the fusions. All different chloroplast types could be established within each combination. Hybrids containing chloroplast from one parent together with mitochondria from the other parent were found in two of the combinations, although the majority of the hybrids had mt-DNA that was altered compared to the parental species. The rearranged mt-DNA found in most hybrids was an effect of the heteroplasmic state following protoplast fusion rather than of the tissue culture methods, since no mt-DNA rearrangements were found in *B. napus* plants regenerated from protoplast culture. The mt-DNA restriction patterns of the hybrids with rearranged mt-DNA indicated that specific regions of the mt-DNA were involved in the rearrangements following protoplast fusion.

Key words: Somatic hybrids – *Brassica* – Phylogeny – Organelle segregation – mt-DNA rearrangements

Introduction

In most higher plants cytoplasmic organelles are maternally inherited. Biparental inheritance of organelles can be achieved by protoplast fusions. Somatic hybridization has enabled the production of plants with new and unique combinations of cytoplasmic organelles. The chloroplast genotype in most regenerated somatic hybrids has been observed to be either of one or the other parental type (reviewed by Maliga and Menczel 1986). Somatic hybrids produced between species that are sexually compatible have revealed random segregation of plastids (Chen et al. 1977; Sidorov et al. 1981; Bonnett and Glimelius 1983; Asahi et al. 1988), while somatic hybrids obtained from fusions made between partially or completely incompatible species have resulted in nonrandom chloroplast segregation (Bonnett and Glimelius 1983; Menczel et al. 1987; Primard et al. 1988; Levi et al. 1988). However, mitochondrial segregation is more difficult to correlate to phylogenetic distance, since mito-

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chondrial DNA (mt-DNA) in somatic hybrids produced both between or within different plant genera frequently have rearranged mt-DNA (reviewed by Hanson et al. 1985). Furthermore, mt-DNA in contrast to chloroplast DNA (cp-DNA) also sometimes undergoes alterations during tissue culture (reviewed by Pelletier 1986), and the use of different culture conditions and fusion techniques, as well as the different methods applied for the mt-DNA analysis, have made comparisons of mt-DNA segregation and rearrangements in different combinations of hybrids or cybrids difficult to perform. However, results have been obtained which demonstrate that the mitochondrial segregation in accordance with chloroplast segregation is influenced by the phylogenetic distance between the fusion partners (Thanh et al. 1988; Bonnett and Glimelius 1990).

In this investigation, we have characterized and compared mt-DNA and cp-DNA isolated from three different combinations of somatic hybrids obtained with reproducible and uniform protoplast fusion and culture systems. All three fusion combinations represent interspecific hybridizations made within the family *Brassicaceae*. The fusions were made between *Brassica campestris* and *B. oleracea*, *B. napus* and *B. nigra*, and between *B. napus* and *Eruca sativa*. The *B. campestris* (+) *B. oleracea* and the *B. napus* (+) *B. nigra* hybrids both represent interspecific hybrids within the same genus but, according to cytogenetic (Röbbelen 1960) and nuclear RFLP (Song et al. 1988) studies, *B. nigra* is less closely related to *B. napus* than *B. campestris* is to *B. oleracea*. The *B. napus* (+) *E. sativa* combination represents an intergeneric hybridization. In addition, mt-DNA was isolated from plants regenerated from protoplasts of *B. napus* in order to determine whether rearrangements were induced by the tissue culture methods.

Materials and methods

Plant material

The investigation was performed on three different combinations of somatic hybrids and on *Brassica napus* plants regenerated from protoplast culture. The *B. campestris* (+) *B. oleracea* combination has been described by Sundberg et al. (1987), the *B. napus* (+) *B. nigra* combination by Sjödin and Glimelius (1989), and the *B. napus* (+) *Eruca sativa* combination by Fahleson et al. (1988). The regeneration of *B. napus* plants from protoplast culture was described by Glimelius (1984). The experimental conditions and plant material used to produce these hybrids were described in the cited publications. In this investigation, the hybrids were designated 1-10 or 1-11 in each fusion combination. These numbers correspond to the nomenclature in the original references as follows: *B. campestris* (+) *B. oleracea* hybrids 1-10 correspond to H12, H1, H14, H16, H4, H19, H5, H6, H7, and H17; *B. napus* (+) *B. nigra* hybrids 1-11 correspond to H6, H11, H12, H13, H14, H16, H18, H21, H22, H26, and H30; and *B. napus* (+) *E. sativa* hybrids 1-10 correspond to J1, J4, J5, J6, J17, J18, J19, J26, J30, and J31.

Organelle DNA analysis

DNA isolation. Mt-DNA was isolated, according to Håkansson et al. (1988), from 5–10 plants obtained from each of the original hybrids by backcrossing with *B. napus* cv Hanna as the pollinator. Mt-DNA of ten *B. napus* plants was studied, each regenerated from a separate protoplast. After self-fertilization of each plant, about ten seedlings were grown and used as material for mt-DNA isolation.

For cp-DNA analysis, total DNA was also isolated from plants of the F_1 generation, according to a modified procedure of Bernatzky and Tanksley (1986). Young leaves, 2–3 g, were frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. Extraction buffer was added to a final volume of 10 ml and the homogenate was transferred to a 50-ml tube. The suspension was adjusted to 1.0% CTAB (hexadecyl trimethyl ammonium bromide, Sigma), 1 *M* NaCl, and 25 m*M* EDTA using 10 ml of lysis buffer (200 m*M* TRIS, 50 m*M* EDTA, 2 *M* NaCl, and 20 g CTAB, pH 8.0). Chloroplasts were lysed by the addition of 2 ml of 10% sarkosyl. The lysate was extracted once with chloroform before the DNA was precipitated with isopropanol. The DNA was dissolved in 750 ml of TE buffer (10 m*M* TRIS, 1 m*M* EDTA, pH 7.5) and treated with 15 µl of RNA:se (2 mg/ml) for 1 h at 60 °C. The DNA was further purified by phenol/chloroform and chloroform extractions, reprecipitated, and finally dissolved in 100–200 µl of TE.

Restriction digests and electrophoresis. Mt-DNA was digested with BamHI and PstI or total DNA with BamHI. The DNA was electrophoresed in 0.5% agarose slab gels (35 mA, 18–20 h). Cp-DNA restriction fragments could be distinguished easily from the nuclear smear on EtBr-stained total DNA gels. Hybrid cp-DNA patterns were compared to purified cp-DNA of the parental species, isolated according to Sundberg et al. (1987), on the same total-DNA gels.

Southern blot and hybridization. Separated mt-DNA fragments were transferred to nylon filters (Pall Biodyne membrane) by the Southern procedure (Maniatis et al. 1982). About 0.1 µg of mt-DNA isolated from the parental species was radiolabeled with 50 µCi (^{32}P) dCTP (Pharmacia oligolabeling kit) and used as probes. Hybridizations were performed in 5 × SSC, 40% formamide, 50 m*M* Na_2PO_4 (pH 6.5), 5 × Denhardt's solution, 0.1% SDS, 10% dextran sulphate, and 0.25% milk powder at 42 °C overnight. Filters were washed twice with 2 × SSC, 0.2% SDS for 5 min at room temperature and twice with 0.7% × SSC, 0.5% SDS for 25 min at 58 °C. Autoradiographs were obtained by exposing Kodak X-Omatic or XAR-15 films to the filters using intensifying screens at −70 °C for 6–40 h. The filters were washed prior to rehybridization with 0.4 *M* NaOH at 42 °C for 5–10 min, followed by two washes in 0.2 × SSC, 0.1% SDS, 0.2 *M* TRIS-HCl, pH 7.5, one at 42 °C for 5–10 min, and the final wash at room temperature for 10 min.

Results

Analysis of parental and hybrid mt-DNA restriction patterns

Mt-DNA from parents and hybrids are shown as autoradiographs in Fig. 1a–c, after probing with mt-DNA of *B. campestris*. Schematic presentations of the BamHI and the PstI banding patterns are shown in Fig. 2a–f. All species could be distinguished by their restriction pattern. The differences in the mt-DNA restriction pat-

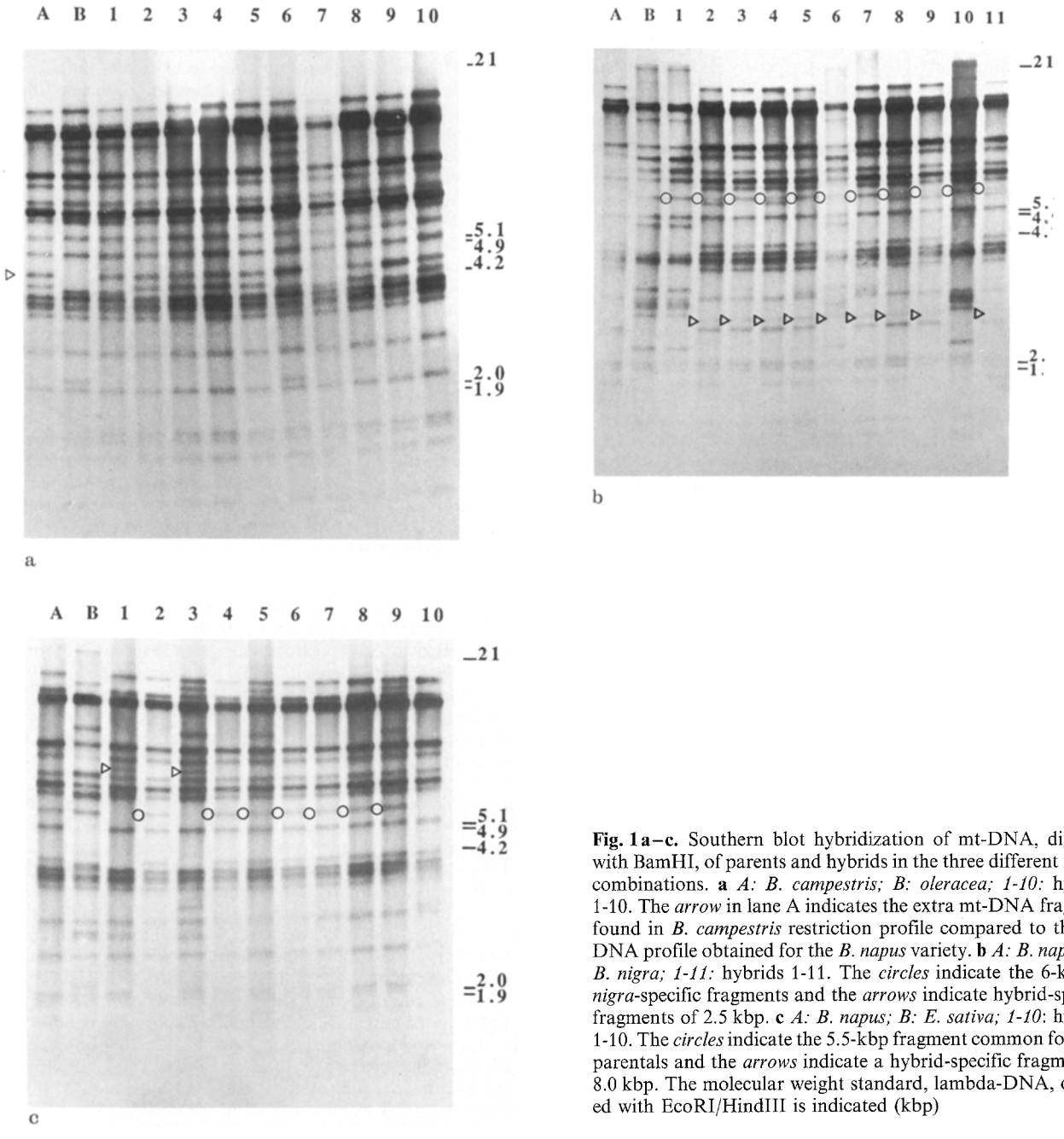

Fig. 1a–c. Southern blot hybridization of mt-DNA, digested with BamHI, of parents and hybrids in the three different fusion combinations. **a** *A: B. campestris; B: oleracea; 1-10:* hybrids 1-10. The *arrow* in lane A indicates the extra mt-DNA fragment found in *B. campestris* restriction profile compared to the mt-DNA profile obtained for the *B. napus* variety. **b** *A: B. napus; B: B. nigra; 1-11:* hybrids 1-11. The *circles* indicate the 6-kbp *B. nigra*-specific fragments and the *arrows* indicate hybrid-specific fragments of 2.5 kbp. **c** *A: B. napus; B: E. sativa; 1-10*: hybrids 1-10. The *circles* indicate the 5.5-kbp fragment common for both parentals and the *arrows* indicate a hybrid-specific fragment of 8.0 kbp. The molecular weight standard, lambda-DNA, digested with EcoRI/HindIII is indicated (kbp)

terns between *B. nigra* and *B. napus* (Figs. 1b and 2b, e) and between *E. sativa* and *B. napus* (Figs. 1c and 2c, f) were larger than the differences between *B. campestris* and *B. oleracea* (Figs. 1a and 2a, d). The filters were rehybridized with total parental mt-DNA from *B. napus*, *B. nigra* and *E. sativa,* respectively. The same restriction patterns were found in all cases (data not shown) and were identical to the restriction profiles obtained when *B. campestris* mt-DNA was used as probe.

B. campestris (+) B. oleracea hybrids. Few differences were observed when comparing the mt-DNA restriction patterns of *B. campestris* and *B. oleracea* after digestion with BamHI (Figs. 1a and 2a) and PstI (Fig. 2d). All hybrids except hybrid 10 had a rearranged mt-DNA compared to the parents, when both restriction patterns were taken into account. The mt-DNA restriction patterns of hybrids 2, 3, 4, 5, and 8 were composed of a complete set of mt-DNA fragments that comigrated with *B. campestris*-specific fragments, together with additional fragments comigrating with *B. oleracea*-specific fragments. Hybrid 6 consisted of a complete set of mt-DNA fragments comigrating with *B. oleracea*-specific fragments, but had also one additional fragment that comi-

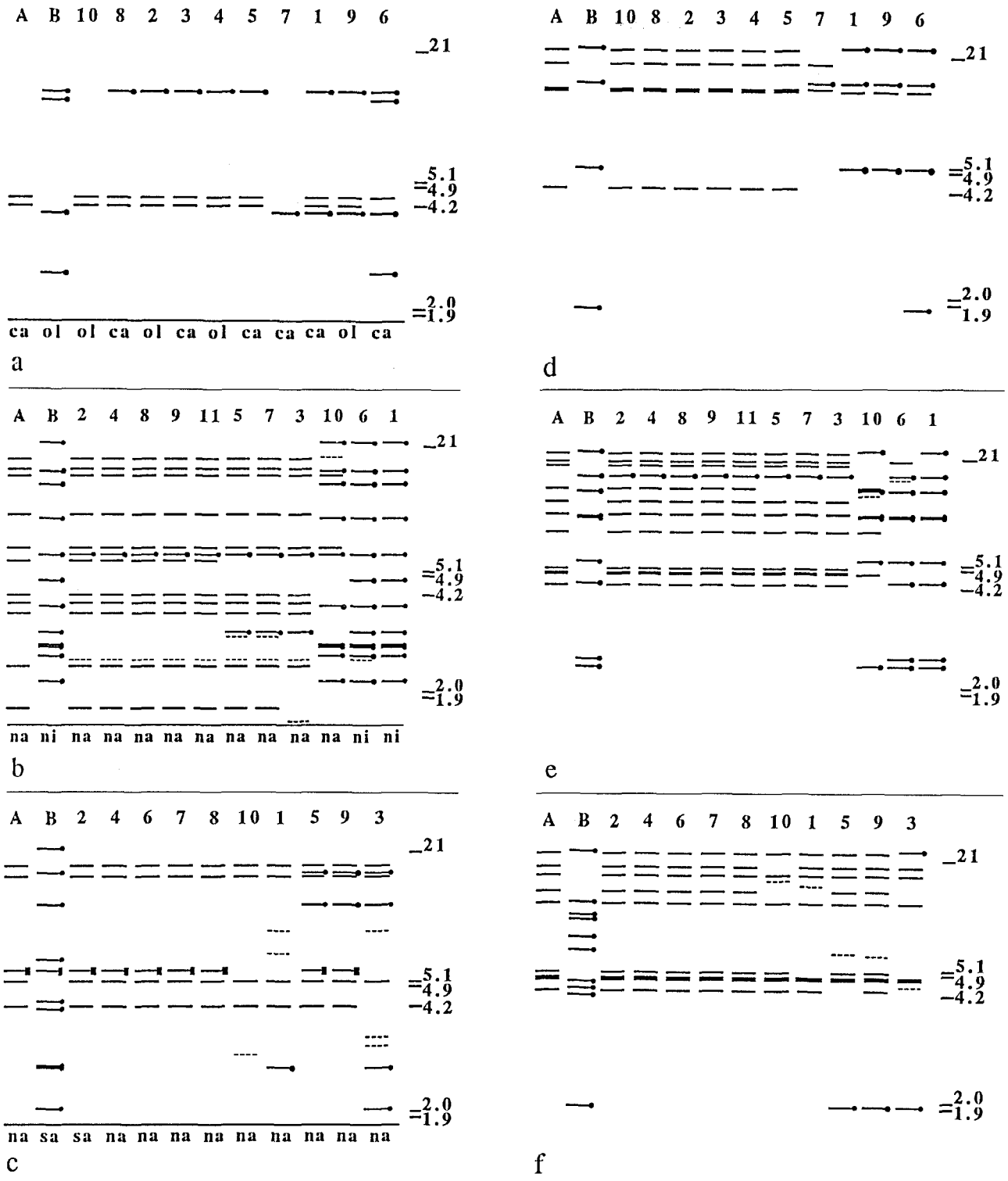

Fig. 2a–f. A schematic presentation of the restriction profiles shown in Fig. 1a–c (**a–c**) and of the Southern blot hybridization of mt-DNA digested with PstI (**d–f**) of parent and hybrids in the three different fusion combinations. All fragments that were common to both parents and all hybrids, according to migration rate, are omitted in the figure. Note that the hybrids have been arranged in different order compared to Fig. 1. The letters given under the *solid line* in the **a–c** denote the chloroplast genotype of the parentals and the hybrids. **a** and **d** *A: B. campestris; B: B. oleracea; 1-10:* hybrids 1-10. **b** and **e** *A: B. napus; B: B. nigra; 1-11:* hybrids 1-11. **c** and **f** *A: B. napus; B: E. sativa; 1-10:* hybrids 1-10. *Solid lines* indicate *B. campestris*- or *B. napus*-specific fragments. The *solid lines* marked with a *solid circle* indicate *B. oleracea-, B. nigra-* or *E. sativa*-specific fragments. *Dashed lines* indicate hybrid-specific fragments. The *solid lines* marked with *solid squares* indicate fragments that were common to both parentals. The molecular weight standard lambda-DNA cut with EcoRI/HindIII is indicated (kbp)

grated with a *B. campestris*-specific fragment. The rearranged mt-DNA in hybrids 1, 7, and 9 was composed of mt-DNA fragments comigrating with parental-specific fragments from both parents, but not the full set of fragments from either of the parents.

B. napus (+) B. nigra hybrids. Out of 20-24 mt-DNA fragments found in the parental restriction patterns, in the BamHI (Figs. 1b and 2b) and PstI (Fig. 2e) profile, respectively, approximately half of the fragments were specific for each parent. The *B. napus* line used in this and in the *B. napus* (+) *E. sativa* combination had a mt-DNA restriction pattern almost identical to *B. campestris*, except for one fragment that was missing after digestion with BamHI and PstI, respectively. In this combination all hybrids except hybrid 1 had a rearranged mt-DNA restriction pattern in both restriction profiles. Hybrid 1 had a mt-DNA restriction pattern identical to *B. nigra*. Hybrids 2, 4, 8, 9, and 11 had identical restriction patterns, which corresponded to the *B. napus* restriction profile, but also contained additional fragments. In the BamHI profile, one of the two additional fragments comigrated with a *B. nigra*-specific fragment of about 6 kbp and the other was a hybrid-specific mt-DNA fragment of about 2.5 kbp (Figs. 1b and 2b). In the PstI profile only one additional fragment, comigrating with a *B. nigra*-specific fragment of about 16 kbp, was discovered in these hybrids (Fig. 2e). In both the BamHI and PstI profiles, hybrids 3, 5, and 7 also had a similar, but incomplete, *B. napus* mt-DNA restriction pattern, together with the fragments comigrating with the *B. nigra* fragment of 6 or 16 kbp, respectively. Moreover, in the BamHI profile these hybrids contained a *B. nigra* fragment of 3.2 kbp and a few novel mt-DNA fragments in addition to the 2.5-kbp hybrid-specific fragment. Hybrids 6 and 10 had a similar mt-DNA restriction pattern to *B. nigra*, but in the PstI profile hybrid 10 contained more fragments comigrating with *B. napus*-specific fragments than hybrid 6. In the BamHI profile, no mt-DNA bands comigrating with *B. napus* fragments were found in hybrid 6.

B. napus (+) E. sativa hybrids. Approximately half of all the *E. sativa* mt-DNA fragments found in the BamHI (Figs. 1c and 2c) and the PstI (Fig. 2f) profiles were species specific. In the PstI profile also about half of all *B. napus* fragments were species specific, while only four fragments were determined as *B. napus*-specific in the BamHI profile. In both the BamHI and the PstI profiles hybrids 2, 4, 6, 7, and 8 had mt-DNA restriction patterns identical to *B. napus*. In hybrids 1, 3, 5, 9, and 10 rearranged mt-DNA was observed. In both the BamHI and the PstI profile hybrid 10 contained mt-DNA similar to *B. napus*, but in the BamHI profile a mt-DNA fragment, which was of about 5.5 kbp and common to both parents, was lost (Figs. 1c and 2c). In addition, hybrid 10 contained novel hybrid-specific fragments in both restriction profiles. The results from both restriction patterns showed that the remaining hybrids 1, 3, 5, and 9 also had mt-DNA similar to *B. napus*, together with one or a few hybrid-specific mt-DNA fragments. In addition, one or a few fragments comigrating with *E. sativa*-specific fragments were found. The 5.5-kbp fragment common to both parents that was absent in hybrid 10 was also absent in hybrids 1 and 3 (Figs. 1c and 2c). In the BamHI profile both hybrids 1 and 3 had a novel fragment of about 8 kbp (Figs. 1c and 2c).

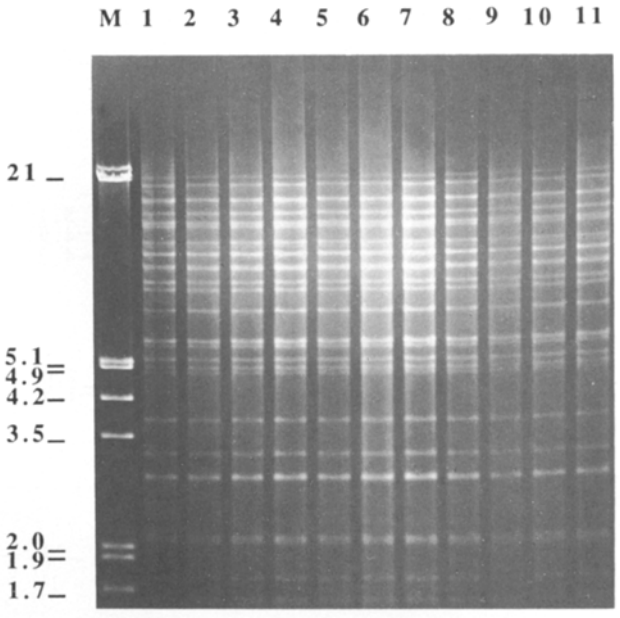

Fig. 3. Restriction pattern of mitochondrial DNA, digested with PstI, isolated from *B. napus* plants grown from seeds (*lane 2*) and from plants regenerated from protoplasts (*lane 3–12*). *Lane 1:* molecular weight standard lambda-DNA cut with EcoRI/HindIII (kbp)

Analysis of mitochondrial DNA restriction pattern in protoplast and seed-derived plants of B. napus

All the *B. napus* plants regenerated from protoplasts had an identical mt-DNA restriction pattern. A comparison of plants regenerated from protoplasts with plants grown from seeds revealed no differences in mt-DNA restriction profiles (Fig. 3). Moreover, several preparations of mt-DNA were made from plants grown from seeds of the parental species used in all combinations, and no variation in mt-DNA restriction patterns was found between different preparations within each species (data not shown).

Chloroplast DNA analysis

Analyses of cp-DNA supplementary to earlier investigations were made in three of the *B. campestris* (+)

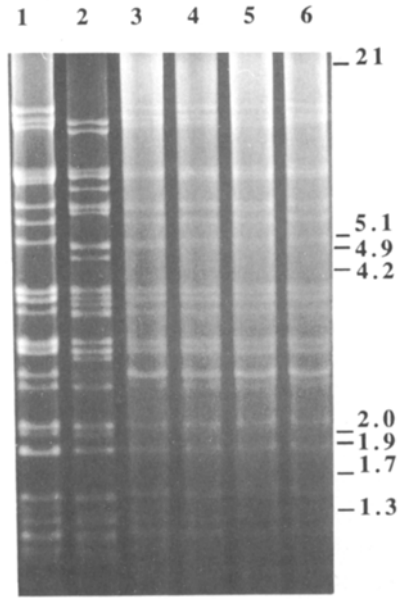

Fig. 4. Restriction pattern of chloroplast and total DNA digested with BamHI. *Lane 1: B. napus* cp-DNA; *2: B. nigra* cp-DNA; *3–6:* total DNA of *B. napus* (+) *B. nigra* hybrids 2, 3, 4, and 5. The molecular weight standard lambda-DNA cut with EcoRI/HindIII is indicated (kbp)

Table 1. A summary of the results obtained after cp-DNA analysis of all hybrids in all combinations. The number of hybrids for each parental chloroplast genotype is given for each hybrid combination

Hybrid combination	Chloroplast genotypes				
	B.cam	*B.ole*	*B.nap*	*B.nig*	*E.sat*
B.cam (+) *B.ole*	5	5			
B.nap (+) *B.nig*			9	2	
B.nap (+) *E.sat*			9		1

B. oleracea hybrids, eight of the *B. napus* (+) *B. nigra* hybrids, and in two of the *B. napus* (+) *E. sativa* hybrids, to determine cp-DNA genotypes of all hybrids for which mt-DNA analyses were performed. All the hybrids had a cp-DNA restriction pattern identical to either one of the parental species. Total DNA digestions from some *B. napus* (+) *B. nigra* hybrids and the restriction patterns of purified cp-DNA from the parents are shown in Fig. 4. The results from earlier cp-DNA investigations and from the analysis performed in this study are summarized in Table 1. In Fig. 2a–c the results of the cp-DNA analyses are given for each hybrid. A combination of chloroplasts from one parent together with unaltered mitochondria from the other parent was found only in *B. campestris* (+) *B. oleracea* hybrid 10 and in the *B. napus* (+) *E. sativa* hybrid 2. In hybrid 10, *B. oleracea* chloroplasts were combined with *B. campestris* mitochondria and in hybrid 2, *E. sativa* chloroplasts were found together with *B. napus* mitochondria. In the *B. napus* (+) *B. nigra* combination, the hybrids contained *B. napus* or *B. nigra* chloroplasts together with a rearranged mt-DNA, except for hybrid 1, which had *B. nigra* chloroplasts together with *B. nigra* mitochondria.

Discussion

Our results demonstrate that rearrangements of mt-DNA were obtained in several hybrids in each of the different combinations. These results are in agreement with earlier studies of mt-DNA in somatic hybrids and cybrids (reviewed by Pelletier 1986). However, Kemble et al. (1988) found no mt-DNA rearrangements in more than 300 *B. napus* hybrid plants. In this investigation we found no mt-DNA rearrangements in the *B. napus* plants regenerated from protoplast culture. Such results have also been reported by Morgan and Maliga (1987) and Kemble et al. (1988). We conclude from our data that the mt-DNA rearrangements found in all three combinations of somatic hybrids resulted from interactions between the parental mt-DNA genomes in the heteroplasmic state following protoplast fusion. We found that all rearranged *B. napus* (+) *B. nigra* and *B. napus* (+) *E. sativa* hybrids contained hybrid-specific mt-DNA fragments, while the rearranged mt-DNA found in the *B. campestris* (+) *B. oleracea* hybrids consisted of a mixture of parental-specific fragments, without any hybrid-specific mt-DNA fragments. Hybrid-specific mt-DNA fragments have been interpreted as a result of intermolecular recombination between the parental mt-DNA genomes in the heteroplasmic state after protoplast fusion (Belliard et al. 1979; Boeshore et al. 1983; Nagy et al. 1983), and intermolecular recombination was confirmed in a *Petunia* somatic hybrid by Rothenberg et al. (1985). In order to explain mt-DNA restriction patterns consisting of a mixture, but not the sum, of parental-specific fragments, an alternative model was suggested by Boeshore et al. (1983), who proposed that separate DNA molecules from the two parentals have assorted in the same mitochondrion following protoplast fusion. Our results indicate that intermolecular recombination has occured in all rearranged *B. napus* (+) *B. nigra* and *B. napus* (+) *E. sativa* hybrids, since they contained hybrid-specific mt-DNA fragments. On the other hand, the absence of hybrid-specific mt-DNA fragments in the rearranged *B. campestris* (+) *B. oleracea* hybrids could result from assortment of parental subcircles. However, due to the fact that the parental mt-genomes were quite similar as judged by the low number of parental-specific fragments, the probability of regenerating hybrid-

specific fragments by intergenomic recombination would be low. Thus, the chance of detecting intermolecular recombination is low.

Biased segregation of organelles could be a result of unequal input of organelles in the original fusion product (Birky 1978). The protoplasts used in the fusions presented here were derived from different tissues. In the *B. campestris* (+) *B. oleracea* combination, all hybrids except hybrid 6 were obtained by fusing *B. campestris* hypocotyl and *B. oleracea* mesophyll protoplasts. In the other two combinations hypocotyl protoplasts were obtained from *B. napus*. However, the cp-DNA analysis showed biased segregation of chloroplasts in the *B. napus* (+) *B. nigra* and the *B. napus* (+) *E. sativa* combinations, favoring the *B. napus* type, while random segregation of chloroplasts was found in the *B. campestris* (+) *B. oleracea* combination. Thus, protoplast type appeared not to have affected chloroplast segregation in the different fusion combinations.

However, it has been found that among vascular plants, more nuclear DNA always involves more plastids per cell (Butterfass 1989). The biased segregation, favoring the *B. napus* chloroplasts, found in two of the combinations in this study, could therefore be due to unequal input of organelles resulting from the fusion between the amphidiploid *B. napus* and the diploid *B. nigra* or *E. sativa*. In contrast, the fusion between the diploid species *B. campestris* and *B. oleracea* resulted in random chloroplast segregation due to equal input of organelles in the hybrid cell. Alternatively, the result from the chloroplast analysis could reflect different plastid replication rates. Analysis of interspecific crossings within *Oenothera* have revealed species-specific plastid multiplication rates (Kirk and Tilney-Bassett 1978). Our results could thus imply that *B. nigra* and *E. sativa* chloroplasts have a lower replication rate compared to *B. napus* chloroplasts, while *B. campestris* and *B. oleracea* chloroplasts seem to have about the same replication rates. Another possibility for biased segregation of organelles in somatic hybrids could be due to elimination of chromosomes from one of the parental species, resulting in a preferential sorting-out of the organelles of that parental type. According to chromosome number and isoenzyme analysis, it was found that many of the *B. napus* (+) *E. sativa* hybrids were asymmetric, and a preferential elimination of *E. sativa* chromosomes was demonstrated (Fahleson et al. 1988). In contrast, most of the *B. napus* (+) *B. nigra* hybrids showed hybrid character for the isoenzymes tested and the expected chromosome number of symmetric hybrids was found (Sjödin and Glimelius 1989). Yet both combinations demonstrated biased chloroplast segregation. Thus, the nuclear constitution of the asymmetric hybrids appears not to have influenced segregation of chloroplasts in the *B. napus* (+) *E. sativa* combination. Nevertheless, even though biased segregation was obtained, all different chloroplast types could be established within each combination.

Similar to the chloroplast segregation, the mt-DNA analysis demonstrated biased segregation, favoring identical or slightly rearranged *B. napus* mitochondrial genomes in both the *B. napus* (+) *B. nigra* and the *B. napus* (+) *E. sativa* combinations. This could be an effect of the different ploidy levels of the fusion partners. However, a preferential segregation of identical or slighlty rearranged mt-DNA obtained from hypocotyl protoplasts was also indicated in the *B. campestris* (+) *B. oleracea* combination. Thus, in contrast to the chloroplast segregation, mitochondrial segregation could have depended on the source of protoplasts used in the fusions. Hypocotyls are composed of young dividing cells that might contain large numbers of mitochondria, and therefore the hypocotyl protoplasts could contribute the majority of mitochondria to the hybrid cell. Moreover, differences in mt-DNA segregation pattern were found between the *B. napus* (+) *E. sativa* and the other two combinations of hybrids. None of the *B. napus* (+) *E. sativa* hybrids had mt-DNA similar or identical to *E. sativa*. In the other combinations, hybrids were obtained that contained mt-DNA similar or identical to either of the parents. The number of hybrids with rearranged mt-DNA was also lower in the *B. napus* (+) *E. sativa* combination in comparison to the other two combinations. These results indicate a correlation of taxonomic differences between the fusion partners and differences of mitochondria replication rate where the mitochondria of the most remote species, *E. sativa*, are the least competetive. The different mt-DNA segregation pattern found in the *B. napus* (+) *E. sativa* combination could also be a result of the preferential elimination of *E. sativa* chromosomes observed in many of these hybrids. However, taking the results together of chromosome number, isoenzyme, and mt-DNA patterns, no correlation could be found between nuclear constitution and mt-DNA type in the *B. napus* (+) *E. sativa* hybrids.

Our hybridization data demonstrated that *B. campestris*, *B. napus*, *B. nigra* and *E. sativa* have mt-DNAs that are very homologous at the primary sequence level, since identical hybridization patterns were obtained regardless of which parental mt-DNA was used as a probe. These results are in accordance with Palmer and Herbon (1988), who reported that mt-DNA in different *Brassica* species has undergone many internal rearrangements, while it is almost homologous in primary mt-DNA sequence. In contrast, at least one-fourth of the fragments observed on an agarose gel containing *N. tabacum* mt-DNA could not be found on the autoradiograph after hybridization with *B. campestris* mt-DNA as probe (data not shown). Even though *B. campestris* is less homologous to the mt-DNA of *N. tabacum* than to the mt-DNA of the other *Brassica* species, intermolecular

recombination could occur in many mt-DNA regions and is not limited to any specific repeated regions. Nevertheless, we found that somatic hybrids within each combination shared several identical rearrangements, which implies that the interaction of the parental mt-genomes was biased to specific regions of the genome. For example, *B. campestris* (+) *B. oleracea* hybrids 1 and 9 had identical rearranged restriction patterns. *B. napus* (+) *B. nigra* hybrids 2, 4, 8, 9, and 11 shared the same rearrangements. In the BamHI profile *B. napus* (+) *E. sativa*, hybrids 1, 3, and 10 had all lost a mt-DNA fragment common to both parents. Identical mt-DNA rearrangements among somatic hybrids of the same combination have also been reported in other investigations (Clark et al. 1986; Kothari et al. 1986; Kemble et al. 1986; Primard et al. 1988; Bonnett and Glimelius 1990). The hybrids presented in this investigation are being evaluated, using gene-specific probes, in order to elucidiate whether specific regions are involved in the rearrangements found in all three different combinations of somatic hybrids.

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References

Asahi T, Kumashiro T, Kubo T (1988) Constitution of mitochondrial and chloroplast genomes in male-sterile tobacco obtained by protoplast fusion of *Nicotiana tabacum* and *N. debneyi*. Plant Cell Physiol 29:43–49

Belliard G, Vedel F, Pelletier G (1979) Mitochondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion. Nature 281:401-403

Bernatzky R, Tanksley SD (1986) Genetics of actin-related sequences in tomato. Theor Appl Genet 72:314–321

Birky CW Jr (1978) Transmission genetics of mitochondria and chloroplasts. Annu Rev Genet 12:471–512

Boeshore ML, Lifshitz I, Hanson MR, Izhar S (1983) Novel composition of mitochondrial gendomes 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

Bonnett HT, Glimelius K (1990) Cybrids of *Nicotiana tabacum* and *Petunia hybrida* have an intergeneric mixture of chloroplasts from *P. hybrida* and mitochondria identical or similar to *N. tabacum*. Theor Appl Genet 79:550–555

Butterfass TH (1989) Nuclear control of plastid division. In: Boffey SA, Lloyd D (eds) Division and segregation of organelles. Society for experimental biology seminar series, vol 35. Cambridge, pp 21–38

Chen K, Wildman SG, Smith HH (1977) Chloroplast DNA distribution in parasexual hybrids as shown by polypeptide composition of fraction I protein. Proc Natl Acad Sci USA 74:5109–5112

Clark E, Schnabelrauch L, Hanson MR, Sink KC (1986) Differential fate of plastid and mitochondrial genomes in *Petunia* somatic hybrids. Theor Appl Genet 72:748–755

Fahleson J, Råhlén L, Glimelius K (1988) Analysis of plants regenerated from protoplast fusions between *Brassica napus* and *Eruca sativa*. Theor Appl Genet 76:507–512

Glimelius K (1984) High growth rate and regeneration capacity of hypocotyl protoplasts in some Brassicaceae. Physiol Plant 61:38–44

Håkansson G, Mark F van der, Bonnett HT, Glimelius K (1988) Variant mitochondrial protein and DNA patterns associated with cytoplasmic male-sterile lines of *Nicotiana*. Theor Appl Genet 76:431–437

Hanson MR, Rothenberg M, Boeshore ML, Nivison HT (1985) Organelle segregation and recombination following protoplast fusion: analysis of sterile cytoplasms. In: Zaitlin M, Day P, Hollaender A (eds) Biotechnology in plant science: relevance to agriculture in the eighties. Academic Press, New York, pp 129–144

Kemble RJ, Barsby TL, Wong RSC, Shepard JF (1986) Mitochondrial DNA rearrangements in somatic hybrid of *Solanum tuberosum* and *Solanum brevidens*. Theor Appl Genet 72:787–793

Kemble RJ, Yarrow SA, Wu SC, Barsby TL (1988) Absence of mitochondrial and chloroplast DNA recombinations in *Brassica napus* plants regenerated from protoplasts, protoplast fusions, and anther culture. Theor Appl Genet 75:875–881

Kirk JTO, Tilney-Bassett RAE (1978) The plastids: their chemistry, structure, growth, and inheritance, 2nd edn. Elsevier North Holland, Amsterdam, pp 433–459

Kothari SL, Monte DC, Widholm JM (1986) Selection of *Daucus carota* somatic hybrids using drug resistance markers and characterization of their mitochondrial genomes. Theor Appl Genet 72:494–502

Levi A, Ridley BL, Sink KC (1988) Biased organelle transmission in somatic hybrids of *Lycopersicon esculentum* and *Solanum lycopersicoides*. Curr Genet 14:177–182

Maliga P, Menczel L (1986) Chloroplast transfer and recombination through protoplast fusion. In: Vasil IK (ed) Cell culture and somatic cell genetics, vol III. Academic Press, Orlando, pp 601–612

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor/NY

Menczel L, Morgan A, Brown S, Maliga P (1987) Fusion-mediated combination of Ogura-type cytoplasmic male sterility with *Brassica napus* plastids using X-irradiated cms protoplasts. Plant Cell Rep 6:98–101

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

Nagy F, Lázár G, Menczel L, Maliga P (1983) A heteroplasmic state induced by protoplast fusion is a necessary condition for detecting rearrangements in *Nicotiana* mitochondrial DNA. Theor Appl Genet 66:203–207

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

Pelletier GR (1986) Plant organelle genetics through somatic hybridization. Oxford Surv Plant Mol Cell Biol 3:97–121

Primard C, Vedel F, Mathieu C, Pelletier G, Chevre AM (1988) Interspecific somatic hybridization between *Brassica napus* and *Brassica hirta* (*Sinapis alba* L.). Theor Appl Genet 75:546–552

Röbbelen G (1960) Beiträge zur Analyse des *Brassica*- genoms. Chromosoma 11:205–228

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

Sidorov VA, Menczel L, Nagy F, Maliga P (1981) Chloroplast transfer in *Nicotiana* based on metabolic complementation between irradiated and iodoacetate-treated protoplasts. Planta 152:341–345

Sjödin C, Glimelius K (1989) *Brassica naponigra*, a somatic hybrid resistant to *Phoma lingam*. Theor Appl Genet 77:651–656

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor Appl Genet 75:784–794

Sundberg E, Landgren M, Glimelius K (1987) Fertility and chromosome stability in *Brassica napus* resynthesised by protoplast fusion. Theor Appl Genet 75:96–104

Thanh ND, Pay A, Smith MA, Medgyesy P, Márton L (1988) Intertribal chloroplast transfer by protoplast fusion between *Nicotiana tabacum* and *Salpiglossis sinuata*. Mol Gen Genet 213:186–190