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A high frequency of intergenomic mitochondrial recombination and an overall biased segregation of *B. campestris* or recombined *B. campestris* mitochondria were found in somatic hybrids made within *Brassicaceae*

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Abstract. Mitochondrial segregation and rearrangements were studied in regenerated somatic hybrids from seven different species combinations produced using reproducible and uniform methods. The interspecific hybridizations were made between closely or more distantly related species within the *Brassicaceae* and were exemplified by three intrageneric, two intergeneric and two intertribal species combinations. The intrageneric combinations were represented by *Brassica campestris* (+) *B. oleracea*, *B. napus* (+) *B. nigra* and *B. napus* (+) *B. juncea* (*tournefortii*) hybrids, the intergeneric combinations by *B. napus* (+) *Raphanus sativus* and *B. napus* (+) *Eruca sativa* hybrids, and the intertribal combinations by *B. napus* (+) *Thlaspi perfoliatum* and *B. napus* (+) *Arabidopsis thaliana* hybrids. In each species combination, one of the two mitochondrial genotypes was *B. campestris* since the *B. napus* cultivar used in the fusions contained this cytoplasm. Mitochondrial DNA (mtDNA) analyses were performed using DNA hybridization with nine different mitochondrial genes as probes. Among the various species combinations, 43–95% of the hybrids demonstrated mtDNA rearrangements. All examined *B. campestris* mtDNA regions could undergo intergenomic recombination since hybrid-specific fragments were found for all of the mtDNA probes analysed. Furthermore, hybrids with identical hybrid-specific fragments were found for all probes except *cox*II and *rrn*18/*rrn*5, supporting the suggestion that intergenomic recombination can involve specific sequences. A strong bias of hybrids having new *atp*A- or *atp*9-associated fragments observed in the intra- and intergeneric combinations could imply that these regions contain sequences that have a high reiteration number, which gives them a higher probability of recombining. A biased segregation of *B. campestris*- or *B. campestris*-like mitochondria was found in all combinations. A different degree of phylogenetic relatedness between the fusion partners did not have a significant influence on mitochondrial segregation in the hybrids in this study.

Key words: Somatic hybrids – *Brassicaceae* – Mitochondrial recombination – Mitochondrial segregation – Phylogenetic differences

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Introduction

Biparental inheritance of organelles can be achieved by protoplast fusion, which has enabled the production of plants with new combinations of cytoplasmic organelles. In view of the complex behaviour of plant mitochondrial genomes (reviewed by Lonsdale 1989) and the fact that in vitro-culture of plant cells may induce mitochondrial DNA (mtDNA) rearrangements (Gengenbach et al. 1981; Kemble and Shepard 1984; Morgan and Maliga 1987), it can be difficult to interpret mtDNA alterations detected in somatic hybrids. Even though mtDNA alterations frequently have been found in somatic hybrids (reviewed by Kumar and Cocking 1987), the knowledge regarding mitochondrial segregation and intergenomic recombination processes in biparental cytoplasms is rather limited. This can partly be explained by the fact that the population of hybrids obtained often has been too small to allow segregation patterns or features important for

intergenomic recombination to be characterized. Furthermore, comparative analyses of hybrids from different fusion experiments have been difficult to perform, owing to large differences in methods used for fusion procedures, in vitro-culture, and mtDNA analysis.

Somatic hybridization within the *Brassicaceae* has been one of the major research areas at our laboratory. During recent years, large numbers of somatic hybrids have been produced by uniform and reproducible methods (Glimelius et al. 1991). In the present study mtDNA analysis of a comparatively large number of somatic hybrids from seven different species combinations was performed. The interspecific hybridizations were made between closely or more distantly related species within the *Brassicaceae* and were exemplified by three intrageneric, two intergeneric and two intertribal species combinations. The intrageneric combinations were represented by *Brassica campestris* (+) *B. oleracea*, *B. napus* (+) *B. nigra*, and *B. napus* (+) *B. juncea(tournefortii)* hybrids, the intergeneric combinations by *B. napus* (+) *Raphanus sativus* and *B. napus* (+) *Eruca sativa* hybrids, and the intertribal combinations by *B. napus* (+) *Thlaspi perfoliatum* and *B. napus* (+) *Arabidopsis thaliana* hybrids. Furthermore, one of the two mitochondrial genotypes in each species combination was *B. campestris* since the *B. napus* cultivar used in the fusions contained this cytoplasm. A comparative study of mtDNA segregation and alterations in these hybrids was carried out in order to investigate whether the different degree of phylogenetic relatedness between the fusion partners was a major factor influencing mitochondrial segregation in biparental cytoplasms. Furthermore, a comparative analysis of this relatively large hybrid material would more evidently demonstrate whether specific mitochondrial regions were involved in intergenomic recombination.

Material and methods

Plant material

The investigation was performed on somatic hybrids obtained from seven different species combinations: *B. campestris* (+) *B. oleracea* (Sundberg et al. 1987), *B. napus* (+) *B. nigra* (Sjödin and Glimelius 1989), *B. napus* (+) *B. juncea(tournefortii)* (Szasz et al. 1991), *B. napus* (+) *Raphanus sativus* (Sundberg and Glimelius 1991 a), *B. napus* (+) *Eruca sativa* (Fahleson et al. 1988), *B. napus* (+) *Thlaspi perfoliatum* (Fahleson et al. 1994), and *B. napus* (+) *Arabidopsis thaliana* (Forsberg et al. 1994). Descriptions of the parental material used for the somatic hybrids can be found in the above-listed references. *B. napus* cv 'Hanna' contained the *B. campestris* cytoplasm and *R. sativus* the male-sterility-inducing 'Ogura' cytoplasm (Ogura 1968), which is derived from *R. sativus* (Makaroff and Palmer 1988). The *B. juncea* cultivar referred to in this study represents a male-sterile, alloplasmic cultivar with the male-sterility-inducing 'Anand' cytoplasm (Rawat and Anand 1979), which originates from *B. tournefortii* (Pradhan et al. 1991; Szasz et al. 1991). Therefore, the *B. juncea* cultivar is designated *B. juncea(tournefortii)*.

Uniform procedures were used for protoplast isolation and for the fusion and culture of hybrid cells (Sundberg et al. 1987). Protoplasts isolated from hypocotyls and leaves were fused using polyethylene glycol. Heterokaryons were enriched by flow cytometry and cell sorting (Glimelius et al. 1986) except for the *B. campestris* (+) *B. oleracea* combination, where mechanical isolation of hybrid cells was used. A culture and regeneration system initially developed for *B. napus* protoplasts was slightly modified so that it could be used for the regeneration of hybrids in all combinations (Glimelius 1984).

The mtDNA analyses were performed on original hybrid plants obtained from *B. napus* (+) *B. juncea(tournefortii)*, *B. napus* (+) *R. sativus*, *B. napus* (+) *A. thaliana*, and the *B. napus* (+) *T. perfoliatum* combinations. In the other three combinations, plants obtained from the first backcross using *B. napus* cv 'Hanna' as the pollinator were used for mtDNA analysis. All hybrids included in this comparative study were regenerated from different calli.

An additional mtDNA study was performed to determine whether the mtDNA segregation process was completed in the original hybrids. For this study mtDNA analyses were performed on first-generation progenies from 15 *B. napus* (+) *B. juncea(tournefortii)* hybrid plants. Up to five plants from each hybrid, or a total of 31 plants, were compared in terms of the patterns of their progenitors.

Mitochondrial DNA analysis

Isolated mtDNA or total DNA digested with *Bam*HI and *Pst*I, respectively, was separated in agarose gel and transferred to nylon filters according to Landgren and Glimelius (1990). Hybridizations were performed with eight heterologous gene probes (Table 1), which contained the *atp*A, *atp*6, *atp*9, *nad*5, *cox*I, *cox*II, *cob*, and *rrn*18/*rrn*5 mitochondrial genes. The probes were kindly provided by Dr. M. R. Hanson, Cornell University and Dr. C. S. Levings III, North Carolina State University. Southern blots and hybridizations were carried out according to Landgren and Glimelius (1990) except that in the hybridization solution 40% formamide was substituted for 6 *M* urea. Chloroplast DNA (cpDNA) isolated from *B. napus*, *B. nigra*, and *E. sativa* was also included in the hybridization experiments to test for cross-hybridization between the mitochondrial gene probes and cpDNA. CpDNA was isolated according to Sundberg et al. (1987).

Results

The DNA hybridization method was sensitive enough to allow mtDNA analysis of hybrids in all species combinations, with the exception of the B. campestris (+) B. oleracea hybrids

In the present investigation eight probes, comprising nine mitochondrial genes, were used for DNA hybridization analysis (Table 1). The results achieved using this procedure were compared with those obtained from the analysis of isolated mtDNA digested with *Bam*HI and *Pst*I, respectively, and stained with ethidium bromide (EtBr) (Landgren and Glimelius 1990). In total, mtDNA of 10 *B. campestris* (+) *B. oleracea*, 11 *B. napus* (+) *B. nigra*, and 10 *B. napus* (+)

Table 1. Summary of the probes and enzymes (B = *Bam*HI, P = *Pst*I) used for mitochondrial DNA analysis of somatic hybrids in seven different species combinations

Hybrid combination	MtDNA probes *atp9* Enzyme		*atp*A		*atp6*		*cox*I		*cox*II		*rrn18/rrn5*		*cob*		*nad5*		Total number of species-specific probes	
	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P
Intrageneric hybrids																		
B. cam (+) *B. ole*	s	s	s	–	–	–	–	–	–	–	–	–	–	–	–	s	2	2
B. nap[a] (+) *B. nig*	s	s	s	s	–	–	s	s	s	s	s	s	–	s	s	s	6	7
B. nap[a] (+) *B. jun(tour)*	s	s	s	s	s	s	s	s	s	s	s	s	–	s	s	s	7	8
Intergeneric hybrids																		
B. nap[a] (+) *R. sat*	s	s	s	s	s	s	s	s	s	s	s	s	–	s	s	s	7	8
B. nap[a] (+) *E. sat*	s	s	s	s	s	s	s	s	s	s	–	s	–	s	–	–	5	7
Intertribal hybrids																		
B. nap[a] (+) *A. tha*	s	s	s	s	s	s	s	s	s	s	s	s	–	s	s	s	7	8
B. nap[a] (+) *T. per*	s	s	s	s	s	s	–	s	s	s	s	s	–	s	s	s	6	8

s indicates that the probes demonstrated a species-specific hybridization pattern while – indicates that no species-specific differences were found

[a] The *B. napus* cultivar contained *B. campestris* cytoplasm

Table 2. Frequencies (%) of somatic hybrids with parental and rearranged mtDNA patterns in seven different species combinations. Hybrids within group 1 had mtDNA patterns consisting of mtDNA fragments identical to both parents (A + B). None of the hybrids had a complete mixture of parental-specific fragments. Group 2 represents hybrids with an incomplete mixture of mtDNA fragments characteristic of both parents together with hybrid-specific fragments (A + B + H). Hybrids with mtDNA patterns containing hybrid-specific fragments together with fragments identical to one or the other parent belonged to group 3 (A + H) and 4 (B + H)

Hybrid combination Parent A B	Number of hybrids	Frequency (%) of hybrids with: Parent A or B specific mtDNA pattern A	B	Rearranged mtDNA patterns Group 1 A+B	2 A+B+H	3 A+H	4 B+H	Sum: groups 1–4
Intrageneric hybrids								
B. cam (+) *B. ole*[b]	10	10	0	90	0	0	0	90
B. nap[a] (+) *B. nig*[b]	22	0	5	0	90	0	5	95
B. nap[a] (+) *B. jun(tour)*	18	22	17	6	33	22	0	61
Intergeneric hybrids								
B. nap[a] (+) *R. sat*[b]	12	33	8	17	25	17	0	59
B. nap[a] (+) *E. sat*[b]	16	56	0	0	31	12.5	0	43.5
Intertribal hybrids								
B. nap[a] (+) *A. tha*	16	50	0	0	37.5	12.5	0	50
B. nap[a] (+) *T. per*	7	57	0	0	43	0	0	43

[a] The *B. napus* cultivar contained *B. campestris* cytoplasm

[b] Combinations included in the study by Sundberg and Glimelius (1991) as referred to in the discussion

E. sativa hybrids were studied, both by analysis of EtBr-stained mtDNA restriction patterns and by DNA hybridization. Only 2 of 9 *B. campestris* (+) *B. oleracea* hybrids that demonstrated rearranged mtDNA according to the EtBr-stained gels showed mtDNA rearrangements with the DNA hybridization method. This large difference in resolution between the methods was expected since for *B. campestris* and *B. oleracea* mtDNA only a small number of mtDNA restriction fragment length polymorphisms (RFLPs) were found using the EtBr-stained gels. The limited number of probes used further restricted the possibilities to find these species-specific mtDNA differences. Therefore, the results obtained from EtBr-stained mtDNA restriction profiles of the 10 *B. campestris* (+) *B. oleracea* hybrids were included in this investigation.

In contrast, all *B. napus* (+) *B. nigra* and *B. napus* (+) *E. sativa* hybrids for which evidence of rearranged mtDNA was obtained using the EtBr-stained gels were also demonstrated to contain mtDNA alterations

using DNA hybridization. Since the methods had the same resolution for the *B. napus* (+) *B. nigra* and *B. napus* (+) *E. sativa* combinations, we assumed that the DNA hybridization method would be sufficiently sensitive for mtDNA analysis of *B. napus* (+) *B. juncea(tournefortii)*, *B. napus* (+) *R. sativus*, *B. napus* (+) *T. perfoliatum*, and *B. napus* (+) *A. thaliana* hybrids, because the numbers of probes showing species-specific differences were about the same in these combinations (Table 1).

None of the mtDNA probes cross-hybridized with cpDNA, except for the *rrn*18/*rrn*5 probe; this probe hybridized with cpDNA-specific fragments. However, these fragments did not show species-specific differences in any of the combinations (data not shown).

Hybrids with mtDNA rearrangements were found in all species combinations

Analysis of the EtBr-stained gels and DNA hybridization patterns revealed that hybrids with mtDNA rearrangements occurred in all species combinations. The rearranged hybrids were divided into four groups according to their mtDNA patterns (Fig. 1, Table 2). The mtDNA of hybrids placed in group 1 contained fragments characteristic of both parents, although they were not the sum of parental-specific fragments. All rearranged *B. campestris* (+) *B. oleracea* hybrids belonged to this group. In contrast, hybrids with a group-1 pattern were absent or uncommon (0–17%) in the other species combinations. Hybrids in group 2 contained an incomplete mix of fragments characteristic of both parents together with new, hybrid-specific fragments. Most of the hybrids (25–90%) with mtDNA alterations were placed in group 2 in all species combinations except for *B. campestris* (+) *B. oleracea*. All hybrids in groups 1 and 2 contained mixtures of fragments characteristic of both parents, although the overall hybridization pattern of each hybrid always resembled one of the parents more than the other. Group 3 and 4 represent hybrids with mtDNA patterns containing fragments identical to *B. campestris* (group 3) or one of the other parents (group 4), together with new, hybrid-specific fragments. Such hybrids were either absent or present at low frequencies (0–22%) in the different combinations. The total frequency (groups 1–4) of hybrids with mtDNA alterations varied between 43% and 95% among the various species combinations.

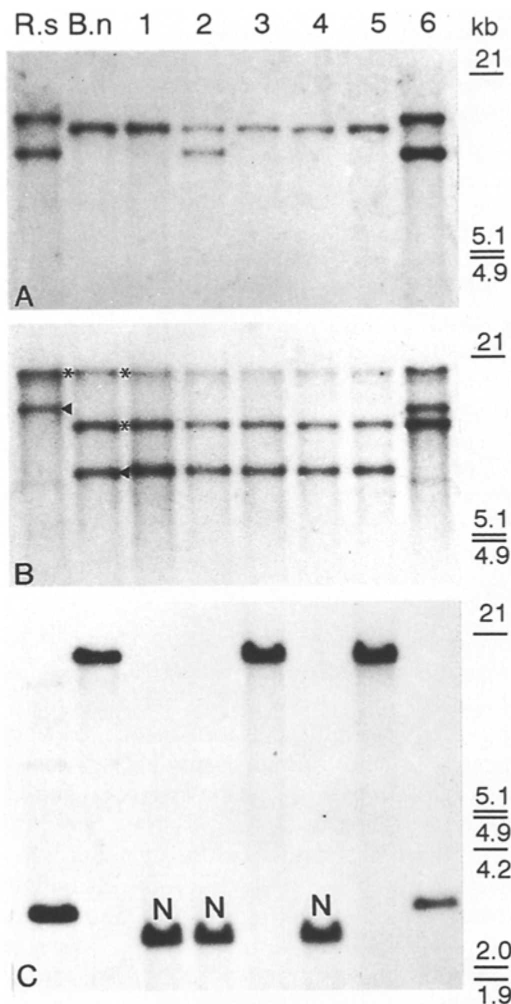

Fig. 1A–C. MtDNA hybridization patterns of *B. napus* (*B.n*), *R. sativus* (*R.s*) and six somatic *B. napus* (+) *R. sativus* hybrids (*1–6*) with four different mitochondrial gene probes. **A** *atp*9, **B** *cox*I and *cob*, where *cob* was hybridized to the filter after *cox*I but without stripping of the *cox*I probe, **C** *atp*A. *Atp*9, *cox*I and *cob* were hybridized to *Pst*I-digested DNA and *atp*A to DNA digested with *Bam*HI. In **B** *cox*I-specific fragments are indicated by *arrows* and *cob*-specific fragments by *asterisks* in the lanes *B.n* and *R.s*. New hybrid-specific fragments are indicated with *N*. Hybrids 3 and 5 have hybridization patterns identical to that of *B. napus*. Hybrids 1 and 4 demonstrate rearranged mtDNA patterns consisting of fragments identical to *B. napus* together with hybrid-specific fragments and represent hybrids of group 3 in Table 2. Hybrid 6 has fragments characteristic of both parents and belongs to hybrids of group 1. Hybrid 2 has fragments identical to both parents together with a hybrid-specific fragment and represents a hybrid of group 2. The molecular weight standard lambda-DNA cut with *Eco*RI/*Hin*dIII is indicated at the *right* (kbp)

Considerable variation was found in the frequency of hybrid-specific fragments associated with different mtDNA regions

Among most of the hybrids showing new, hybrid-specific mtDNA fragments, these fragments were associated with the *atp*9 and *atp*A genes (Table 3). Between 40% and 86% of the intrageneric and intergeneric hybrids had hybrid-specific fragments that hybridized to the *atp*9 probe. Among these combinations, there were similar frequencies of hybrids with hybrid-

Table 3. Frequencies (%) of somatic hybrids demonstrating hybrid-specific mtDNA fragments for each one of eight different probes comprising nine mitochondrial genes in seven different species combinations

Hybrid combination	Number of hybrids with new hybrid-specific fragments	MtDNA probes *atp*9	*atp*A	*cox*I	*cox*II	*rrn*18/*rrn*5	*cob*	*nad*5	*atp*6
Intrageneric hybrids									
B. cam (+) *B. ole*	0	0	0	0	0	0	0	0	0
B. nap[a] (+) *B. nig*	21	86	29	5	0	5	0	0	0
B. nap[a] (+) *B. jun(tour)*	10	40	80	0	0	0	0	10	40
Intergeneric hybrids									
B. nap[a] (+) *R. sat*	5	40	60	60	0	0	0	0	0
B. nap[a] (+) *E. sat*	7	43	71	0	0	0	0	29	0
Intertribal hybrids									
B. nap[a] (+) *A. tha*	8	12.5	12.5	62.5	12.5	25	37.5	25	0
B. nap[a] (+) *T. per*	3	100	67	100	0	0	67	67	67

[a] The *B. napus* cultivar contained *B. campestris* cytoplasm

specific fragments related to *atp*A (29–80%). None of the hybrid-specific fragments within the intrageneric or intergeneric species combinations were associated with the *cox*II or the *cob* genes. Similarly only 1 *B. napus* (+) *B. nigra* hybrid had a hybrid-specific fragment related to the *rrn*18/*rrn*5 probe.

The distribution of hybrid-specific fragments associated to the different gene probes was more skewed in the intrageneric and intergeneric species combinations than in the intertribal hybrids. In the *B. napus* (+) *A. thaliana* combination, we found hybrid-specific fragments related to all probes except *atp*6-associated fragments. A similar distribution was found in the *B. napus* (+) *T. perfoliatum* combination, where hybrid-specific fragments related to *cox*II and *rrn*18/*rrn*5 were absent. Furthermore, as shown in Fig. 1, hybrids with identical mtDNA rearrangements were found in all combinations. Within each combination, between 2 and 15 hybrids had identical hybrid-specific fragments for at least one of the probes. Hybrids with identical hybrid-specific fragments were found for all probes except *cox*II and *rrn*18/*rrn*5.

MtDNA analysis of original B. napus (+) B. juncea(tournefortii) hybrids and their progenies

The hybrids investigated in the present study were either original hybrid plants or first-generation hybrid progenies. In order to be able to study reasonably large hybrid populations, we performed mtDNA analyses on original hybrid plants in combinations of distantly related species since these hybrids often had reduced fertility or were sterile. Provided that the mtDNA segregation process had been completed in the original hybrid plants a comparative study could be made of mtDNA segregation and alterations in the different hybrid combinations. MtDNA analyses were therefore performed on 15 original *B. napus* (+) *B. juncea(tournefortii)* hybrids and their progenies. Only 1 out of 31 of the *B. napus* (+) *B. juncea(tournefortii)* hybrid progenies had a mtDNA pattern that deviated from that of the original hybrid parent presented in Table 2 (data not shown). This highly stable inheritance of mtDNA patterns made comparisons of mtDNA segregation and alterations among the different hybrid combinations possible.

Biased segregation of B. campestris mtDNA or mtDNA similar to B. campestris was found within all species combinations

The segregation of parental genotypes was biased in both intergeneric and intertribal hybrid combinations, favouring the *B. campestris* type (Table 2). The frequencies of hybrids with parental mitochondria in these combinations varied between 41% and 57%, of which the majority (87–100%) represented hybrids with *B. campestris* mitochondria. Since only 5–39% of the hybrids in the intrageneric combinations had mitochondria of the parental type, it was not possible to distinguish any clear trends in mitochondrial segregation patterns for parental types in these combinations.

Biased segregation favouring mitochondria similar to *B. campestris* was found among all hybrids demonstrating mtDNA rearrangements. This becomes very clear if the grouping in Table 2 is altered. Among hybrids with mtDNA patterns containing an incomplete mix of parental-specific fragments, with or without hybrid-specific fragments, i.e. groups 1 and 2 in Table 2, two main types could be distinguished

Table 4. Frequencies (%) of somatic hybrids with rearranged mtDNA patterns similar to one (**A**) or the other (**B**) parent in seven different species combinations. The rearranged hybrids had either an incomplete mix of parental-specific fragments, with or without new hybrid-specific fragments (**A** + B + H or **B** + A + H), or fragments identical to one of the parents together with hybrid-specific fragments (A + H or B + H)

Hybrid combination Parent A	B	Number of hybrids with mt DNA rearrangements	Frequencies of hybrids with mtDNA rearrangements having a mtDNA similar to Parent A **A** + B + H	A + H	Parent B **B** + A + H	B + H	Sum of **A** + B + H and A + H	**B** + A + H and B + H
Intrageneric hybrids								
B. cam (+)	*B. ole*	9	67	0	33	0	67	33
B. nap[a] (+)	*B. nig*	21	95	0	0	5	95	5
B. nap[a] (+)	*B. jun(tour)*	11	45	36	18	0	81	18
Intergeneric hybrids								
B. nap[a] (+)	*R. sat*	7	43	28	29	0	71	29
B. nap[a] (+)	*E. sat*	7	71	29	0	0	100	0
Intertribal hybrids								
B. nap[a] (+)	*A. tha*	8	62.5	25	12.5	0	87.5	12.5
B. nap[a] (+)	*T. per*	3	67	0	33	0	67	33

[a] The *B. napus* cultivar contained *B. campestris* cytoplasm

(Table 4): those with recombined mtDNA similar to *B. campestris* (class **A** + B + H) and those with recombined mtDNA similar to the other parent (class **B** + A + H) (Table 4). An overview of the mtDNA patterns of the new groups, together with groups 3 and 4 from Table 2, is presented in Table 4. Between 67% and 100% of the rearranged hybrids had a mtDNA similar to that of *B. campestris.*

Discussion

The mtDNA alterations recovered in the current study indicated that intergenomic recombination was induced by protoplast fusion. Besides the rearranged mtDNA patterns found in *B. campestris* (+) *B. oleracea* hybrids, most of the rearranged hybrids had mtDNA patterns containing new, hybrid-specific fragments together with fragments characteristic of both parental species. The mtDNA rearrangements found in the hybrids were not due to in vitro-culture-induced alterations of the *B. campestris* mt genome. In a previous study, no mtDNA rearrangements were found in *B. napus* plants regenerated through protoplast culture (Landgren and Glimelius 1990). Neither can in vitro-culture-induced mtDNA rearrangements of the other parental genomes plausibly explain the alterations found in the hybrids since all rearranged hybrids contained mtDNA fragments identical to *B. campestris,* with the exception of one rearranged *B. napus* (+) *B. nigra* hybrid. It is unlikely that such culture-induced changes have led to the regeneration of fragments characteristic of *B. campestris* in all except 1 hybrid. Hence, the mtDNA rearrangements found in the hybrids were induced by the heteroplasmic stage following protoplast fusion.

Segregation analysis of parental and rearranged mitochondrial types clearly demonstrated a biased segregation that favoured *B. campestris* or *B. campestris*-like mitochondria in all combinations. *B. campestris* or *B. campestris*-like mitochondria was equally favoured in all combinations. In contrast, Sundberg and Glimelius (1991b) found that when they compared six different fusion combinations (four of those are represented in this investigation see Table 2) chloroplast segregation generally occurred more randomly in hybrid populations obtained from fusions of the more closely related species. It has also been recently demonstrated that chloroplast transmission in cybrids is influenced by the degree of phylogenetic relatedness between the fusion partners (Perl et al. 1991). However, no notable difference regarding the mitochondrial segregation pattern was found in the present investigation. Thus, the different degree of phylogenetic relatedness between the fusion partners did not have a significant influence on mitochondrial segregation.

The overall biased segregation of *B. campestris* or *B. campestris*-like mitochondria could be due to several factors. Differences in the input of mitochondrial genomes could influence the mitochondrial segregation pattern. The fusions presented in this investigation were made between hypocotyl and mesophyll protoplasts with *B. campestris* mitochondria exclusively donated from hypocotyl cells. The influence of cell type on organelle segregation has been studied in hypocotyl-mesophyll fusions by Walters et al. (1993). They found that both mitochondrial and chloroplast segregation were biased, but these biases were generally unaffected

by protoplast type. Similarly, Sundberg et al. (1991) found a biased segregation of *B. napus* (i.e. *B. campestris*) chloroplasts irrespective of from which cell type the chloroplasts were donated. Sundberg et al. (1991) proposed that the biased chloroplast segregation obtained in reciprocal hypocotyl-mesophyll fusions was an effect of ploidy differences between the fusion partners. Nuclear DNA content and cell size both influence plastid number and chloroplast DNA content (Butterfass 1989). Consequently, differences in ploidy level between the fusion partners could cause an unequal input of chloroplasts in the biparental cytoplasm following fusion. Similarly, the biased segregation of *B. campestris* or *B. campestris*-like mitochondria in this investigation could be an effect of ploidy differences between the fusion partners. Six out of the seven fusion combinations were combinations between the allotetraploid *B. napus* and diploid relatives to *B. napus*. However, the biased mitochondrial segregation pattern found among the *B. campestris* (+) *B. oleracea* hybrids does not fit into that explanation since these hybrids were made between two diploid species.

Conditions which also might favour *B. campestris* or *B. campestris*-like mitochondria could be the culture and regeneration system. No deliberate selection was made during the culture and regeneration of the hybrids in the present study. However the culture and regeneration media were developed for the *B. napus* cultivar, and it cannot be excluded that this influenced mitochondrial segregation. Another factor affecting mitochondrial segregation in biparental cytoplasms could be nuclear-mitochondrial interactions. Several examples of cytoplasmic male sterility is thought to be caused by nuclear-mitochondrial incompatibility (reviewed by Braun et al. 1993). Extensive nuclear influence on mitochondrial transcription and genome structure has also been demonstrated in alloplasmic *Nicotiana* cultivars (Håkansson and Glimelius 1991). The biased segregation of the *B. campestris* or *B. campestris*-like mitochondria found in this study might indicate nuclear mitochondrial interactions favouring *B. campestris* mitochondria. During their study of chloroplast segregation, Sundberg and Glimelius (1991b) also investigated the nuclear composition of the hybrids. They found that chromosome elimination increased in the hybrids obtained from the most distantly related species combinations and that the diploid genomes were preferentially eliminated while the nuclear genome of *B. napus* was retained. However, increasing frequencies of asymmetric hybrids in the combinations made between more distantly related species did not have a significant influence on mitochondrial segregation in the present investigation, which possibly indicates that neither of the mitochondrial genomes are as competitive with their own nucleus as *B. campestris* is with the *B. napus* nucleus. The replication and transmission rate of mitochondrial genomes from the different species could also differ due to inherent mitochondrial differences. In yeast, differences in mtDNA replication rates have been found between mitochondrial mutants (reviewed by Dujon 1981). Some of the mutants which could replicate faster than their progenitors were found to contain a higher number of *ori/rep* sequences. In addition, intergenic sequences flanking the *ori/rep* sequences were also found to modulate the efficiency of mtDNA transmission to the progeny (Piskur 1988, 1989; Rayko et al. 1988). The rapid change in the linear-sequence order in the *Brassica* mitochondrial genomes (Palmer and Hebron 1988) might have altered the number of origins of replication sequences (i.e. *ori/rep* sequences) or rearranged intergenic flanking sequences. These changes could result in inherent differences influencing the replication and transmission rate of the mitochondrial genomes from different species.

High frequencies of somatic hybrids with mtDNA alterations were found in each of the different species combinations. In addition, hybrids with identical mtDNA rearrangements were found for all probes except *cox*II and *rrn*18/*rrn*5. Hybrids displaying identical rearrangements have been reported in several studies (Kemble et al. 1986; Kothari et al. 1986; Primard et al. 1988; Temple et al. 1992). It has been suggested, in accordance with the model of the organization of the mitochondrial genome that involves intramolecular recombination between repeated sequences, that sites involved in intergenomic recombination also might be limited to certain repeats (Clark et al. 1986). Recently Makaroff et al. (1991) showed that repeated mtDNA sequences, other than those involved in the intragenomic recombination, were associated with recombination processes. However, the frequencies of somatic hybrids demonstrating hybrid-specific mtDNA fragments for each one of the eight different probes differed among the combinations (Table 3). Most of the intrageneric and intergeneric hybrids were found to have new, hybrid-specific fragments associated to the *atp*9 and *atp*A gene, respectively. In contrast, there was not such a strong bias towards hybrids having new *atp*A- or *atp*9-associated fragments in the intertribal combinations. Due to the rapid change in the linear-sequence order in the *Brassica* mitochondrial genomes (Palmer and Hebron 1988) recombination between more distantly related genomes might be more restricted to the coding sequences and their immediate flanking sequences. Recombination can be expected to occur between any of the homologous sequences that correspond to the even distribution of hybrid-specific fragments found in the intertribal combinations. In contrast the strong bias of hybrids having new *atp*A- or *atp*9-associated fragments observed in the intra- and intergeneric combina-

tions imply that these regions contain sequences with a higher probability to recombine. Recombination could preferentially occur between regions containing a high number of reiterated sequences. Individual repeated sequences have been found near several mitochondrial genes (reviewed by André et al. 1992). However, whether any of these sequences or other repeated sequences are also reiterated in the *atp*9 and *atp*A regions studied in this investigation remains to be determined.

To conclude, an overall biased segregation of *B. campestris* or *B. campestris*-like mitochondria was found in all combinations. However, the different degree of phylogenetic relatedness between the fusion partners did not have a significant influence on mitochondrial segregation in this study. All of the examined *B. campestris* mtDNA regions were able to undergo intergenomic recombination, and hybrid-specific fragments were found for all of the mtDNA probes tested. The fact that hybrids with identical mtDNA rearrangements were observed within all species' combinations in this study strengthens the suggestion that intergenomic recombination involves repeated sequences. Moreover, the results of this study imply that the *B. campestris* mitochondrial genome contains such sequences within several regions and that some of these sequences may have a high reiteration number.

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