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Location, identity, amount and serial entry of chloroplast DNA sequences in crucifer mitochondrial DNAs

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Abstract. Southern blot hybridization techniques were used to examine the chloroplast DNA (cpDNA) sequences present in the mitochondrial DNAs (mtDNAs) of two *Brassica* species (*B. campestris* and *B. hirta*), two closely related species belonging to the same tribe as *Brassica* (*Raphanus sativa, Crambe abyssinica*), and two more distantly related species of crucifers (*Arabidopsis thaliana, Capsella bursa-pastoris*). The two *Brassica* species and *R. sativa* contain roughly equal amounts (12–14 kb) of cpDNA sequences integrated within their 208–242 kb mtDNAs. Furthermore, the 11 identified regions of transferred DNA, which include the 5′ end of the chloroplast *psa*A gene and the central segment of *rpo*B, have the same mtDNA locations in these three species. *Crambe abyssinica* mtDNA has the same complement of cpDNA sequences, plus an additional major region of cpDNA sequence similarity which includes the 16S rRNA gene. Therefore, except for the more recently arrived 16S rRNA gene, all of these cpDNA sequences appear to have entered the mitochondrial genome in the common ancestor of these three genera. The mitochondrial genomes of *A. thaliana* and *Capsella bursa-pastoris* contain significantly less cpDNA (5–7 kb) than the four other mtDNAs. However, certain cpDNA sequences, including the central portion of the *rbc*L gene and the 3′ end of the *psa*A gene, are shared by all six crucifer mtDNAs and appear to have been transferred in a common ancestor of the crucifer family over 30 million years ago. In conclusion, DNA has been transferred sequentially from the chloroplast to the mitochondrion during crucifer evolution and these cpDNA sequences can persist in the mitochondrial genome over long periods of evolutionary time.

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Introduction

The idea of organelle genomes as discrete, separate entities has recently been replaced by the idea of a more dynamic relationship existing between the genomes of different cellular organelles. In plants, in particular, several studies have presented evidence that interorganellar DNA transfer has been both extensive and frequent in the evolution of nuclear, chloroplast, and mitochondrial genomes. The term "promiscuous DNA" (Ellis 1982) has been coined to describe DNA sequences that are present in more than one cellular genome. Stern and Lonsdale (1982) first demonstrated the presence of a 12 kb DNA sequence in both the chloroplast and mitochondrial genomes of maize. Since then, the presence of shared DNA sequences in the chloroplast and mitochondrial genomes of other angiosperms has been shown to be a general phenomenon (Stern et al. 1983; Stern and Palmer 1984, 1986). In addition, evidence of DNA transfer has been documented between the mitochondrion and nucleus (Kemble et al. 1983; Schuster and Brennicke 1987) and the chloroplast and nucleus (Timmis and Scott 1983; Scott and Timmis 1984; Ayliffe et al. 1988).

Evolutionary and functional considerations support the idea that the direction of sequence movement between cytoplasmic genomes has been from the chloroplast to the mitochondrion. DNA sequences shared between the chloroplast and mitochondrial genomes are highly conserved both in nucleotide sequence and in arrangement within the chloroplast genome (Palmer and Thompson 1982) and in most cases are probably tran-

scribed and functional in the chloroplast. On the other hand, these shared sequences are randomly distributed within the mitochondrial genomes of different plants (Stern and Palmer 1984), and, with only a few exceptions known to date (Marechal et al. 1987; Wintz et al. 1988), appear not to be transcribed (Makaroff and Palmer 1987). It appears therefore that transfer has occurred from the highly conserved chloroplast genome (Palmer and Thompson 1982; Palmer 1985) into the more variable mitochondrial genome (Ward et al. 1981). Transfer of foreign DNA sequences into the chloroplast genome has not been shown. This may be due to the compact nature of the chloroplast genome, which contains very little untranscribed DNA (Ohyama et al. 1986; Shinozaki et al. 1986). Integration of foreign DNA into the chloroplast DNA (cpDNA) molecule is likely to disrupt an essential gene function and therefore would be selected against.

Although it is known that a number of cpDNA sequences have been transferred to the mitochondrion, a quantitative determination of the amount of cpDNA sequences present in mitochondrial DNA (mtDNA) has not been carried out. Likewise, the timing of inter-organellar DNA transfer is unknown. The linear order of cross-hybridizing sequences between cpDNA and mtDNA is different in the two cases examined (Stern and Palmer 1986; Siculella and Palmer 1988). What is not known is whether DNA transfer occurred once or a few times, followed by multiple rearrangements of the mitochondrial genome, or whether many separate transfer events have led to the dispersal of cpDNA sequences within the mitochondrial genome. The growing literature on the prevalence of rearrangements within plant mitochondrial genomes (reviewed by Palmer 1985; Lonsdale 1988) certainly strengthens the case for the first hypothesis.

In the present work we quantitatively determine the amount of DNA sequences shared between the chloroplast genome and the mtDNAs of six members of the crucifer family (Brassicaceae). We show that DNA transfer between organelles has occurred sequentially and in addition we identify precise regions of the chloroplast genome that are present in the mitochondrial genomes of these species.

Materials and methods

Mitochondrial and chloroplast DNAs were isolated from the green leaves of turnip (*Brassica campestris* cv. Purple top white globe), white mustard (*B. hirta*, syn. *Sinapis alba*, USDA PI 195, 922), radish (*Raphanus sativa* cv. Scarlet Knight), *Crambe abyssinica*, shepherd's purse (*Capsella bursa-pastoris*), and *Arabidopsis thaliana* (cv. Columbia wild-type) by standard procedures (Kolodner and Tewari 1972; Palmer 1986). Each DNA isolation was from six large flats of six-week old plants (approx. 50 plants per flat). *Arabidopsis thaliana* seeds were obtained from Chris Somerville, Michigan State University, all other crucifer seeds were provided by Paul Williams of the Crucifer Genetics Co-operative, Madison, Wisconsin. A *B. campestris* cpDNA clone bank was constructed by cloning PstI-, SacI- and PstI/SacI-digested cpDNA fragments into the appropriate sites in the pUC12 cloning vector. Two additional clones, P12.3 and P15.1, were kindly provided by Dr. G. Link. Subcloning of chloroplast gene-specific regions was carried out using the vectors pUC12 (*rpo*B, 1.1 kb BamHI fragment), pIC20R (3′ *rbc*L, 0.8 kb HindIII-BamHI fragment; *rbc*L internal, 1.1 kb PstI-HindIII fragment; 5′ *rbc*L, 0.7 kb XbaI-PstI fragment) and pUC8 (*psa*A, 2.4 kb BamHI fragment). 3′ and 5′ regions of *psa*A were obtained by cutting the BamHI 2.4 kb clone with the restriction enzyme KpnI and isolating the resulting two fragments from a 1% low-melting agarose gel. *Brassica campestris* mtDNA clones used were from the clone bank of Palmer and Shields (1984). Restriction endonuclease digestions, gel electrophoresis, transfer of DNA fragments to Zetabind filters, nick-translations, cpDNA cloning in plasmid vectors, and isolation of plasmid DNA were as described (Palmer 1986). Following hybridization, and prior to autoradiography, filters were washed in 2 x SSC (0.3 M NaCl, 30 mM trisodium citrate) and 0.5% SDS (lauryl sulphate sodium salt) at 65 °C. Filters were stripped of hybridized probe before rehybridization by incubation in 0.5 M NaOH at 50 °C for 1 h. Densitometric determinations were made using an LKB Ultrascan XL Laser Densitometer.

Results

Presence of cpDNA sequences in crucifer mtDNAs

To identify cpDNA sequences in crucifer mtDNAs we constructed a clone bank containing all but 6 kb of the 151 kb *B. campestris* chloroplast genome. Cloned cpDNA fragments were hybridized to blots containing PstI, SalI, BglI, and KpnI digests of mtDNAs from two species of *Brassica* (*Brassica hirta, B. campestris*), two closely related species (*Raphanus sativa* and *Crambe abyssinica*) belonging to the same tribe as *Brassica*, and two more distantly related species (*Capsella bursa-pastoris* and *Arabidopsis thaliana*) belonging to different tribes in the crucifer family (see Fig. 1 for a phylogenetic tree depicting the relationships among these six crucifers). We chose these species so that we might be able to estimate at what evolutionary time intervals specific cpDNA sequences entered the mitochondrial genome. The absence of significant intraspecific variation in *Brassica* mtDNAs (Palmer 1988) gives us confidence that observed genus- and tribal-specific differences in cpDNA-derived sequences within mtDNAs are not the result of population variation within a species. To discriminate between actual DNA sequences shared between the chloroplast and mitochondrial genomes and hybridization signals attributable to cpDNA contamination of the mtDNA preparations, cpDNA prepared from the same species and digested with the same re-

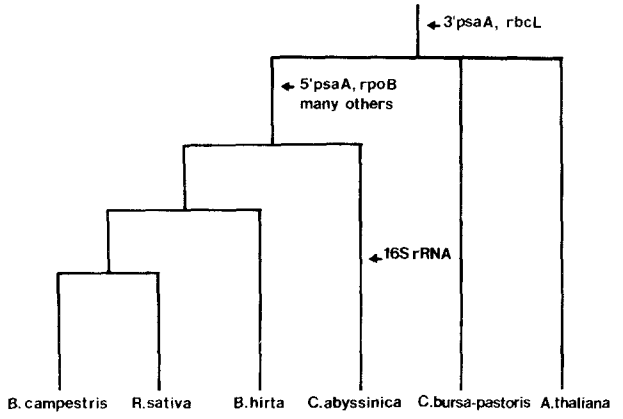


Fig. 1. Phylogenetic tree depicting the relationships among the six crucifer species. The relationship between *B. campestris, R. sativa* and *B. hirta* is based on cpDNA data (Palmer et al. 1983b). The broader relationship between tribes is based on morphological data (Heywood 1985). *Arrows* indicate the relative times of entry of certain cpDNA regions into the mitochondrial genome

striction enzyme was included as a comparative control in lanes adjacent to each mtDNA track. In this way we could identify hybridization bands that were due to contaminating cpDNA and disregard them.

The majority of the cpDNA clones hybridized to regions within all the mtDNAs examined; only six cpDNA clones have no detectable sequence similarity to any of the mtDNAs (Fig. 2). However, the relative intensities of the hybridization signals obtained varied among the six species. A SacI clone of 9.8 kb (S9.8) and a PstI clone of 12.3 kb (P12.3) hybridized very strongly to the mtDNAs of the two *Brassica* species, *R. sativa*, and *Crambe abyssinica*, but only weakly, or not at all, to the mtDNAs of *Capsella bursa-pastoris* and *A. thaliana*. P2.2, which spans one junction of the chloroplast inverted repeat and large single copy region, has a high degree of sequence similarity only to the mtDNAs of *Capsella bursa-pastoris* and *A. thaliana*. S3.5 and PstI-SacI 5.8 (PS5.8), two clones from within the chloroplast inverted repeat region, hybridized very strongly to the mtDNA of *Crambe abyssinica* relative to the mtDNAs of the other five species.

Quantitation of the amount of shared sequences between cpDNA and mtDNA

An estimate of how much cpDNA is present in each of the six mtDNAs was obtained by carrying out quantitative hybridization experiments. Approximately equimolar amounts of cpDNA (from *B. campestris*) and mtDNA (from all six species) were digested with PstI, Southern-blotted and probed with *B. campestris* cpDNA clones. The intensities of the hybridization signals obtained were measured by densitometric scanning of autoradiograms. The approximate length of each shared DNA sequence was estimated by dividing the densitometrically-measured intensity of hybridization to a mtDNA band by the intensity of cpDNA self-hybridization, and then multiplying this fraction by the length

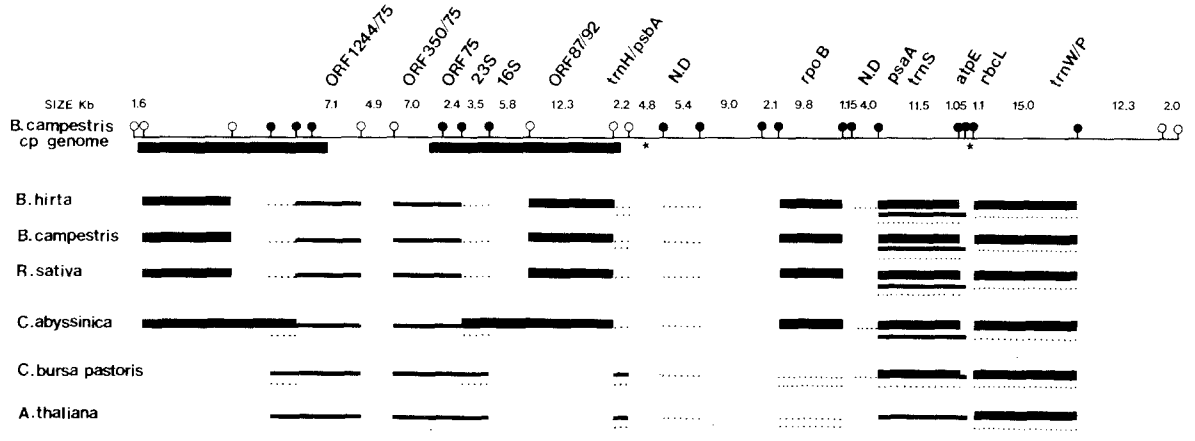
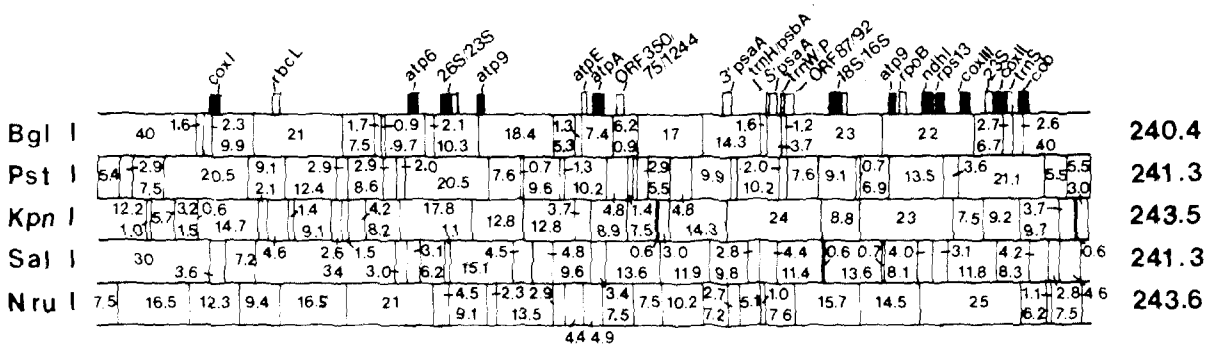
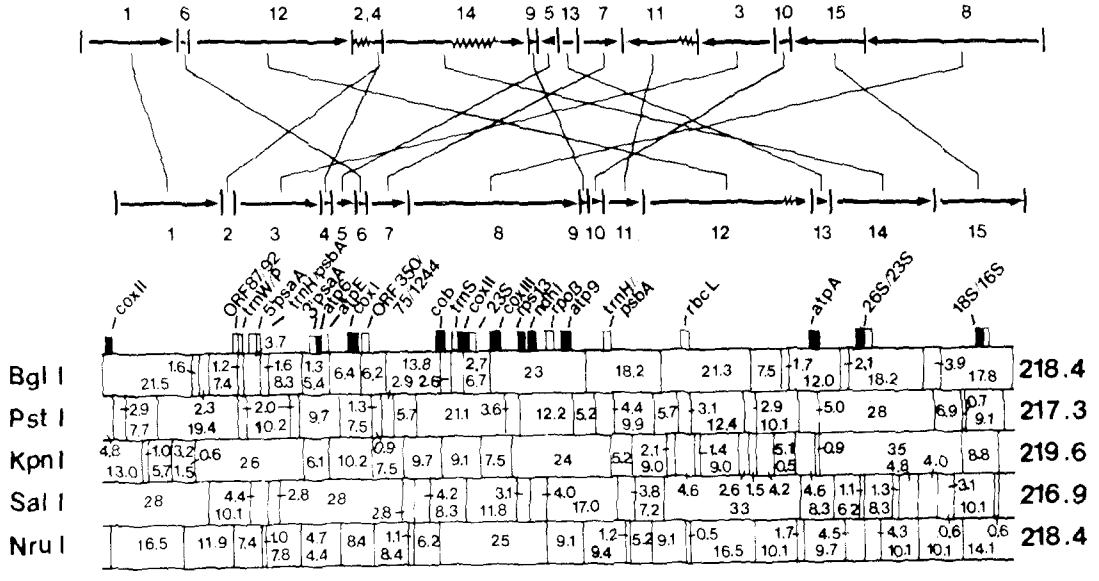


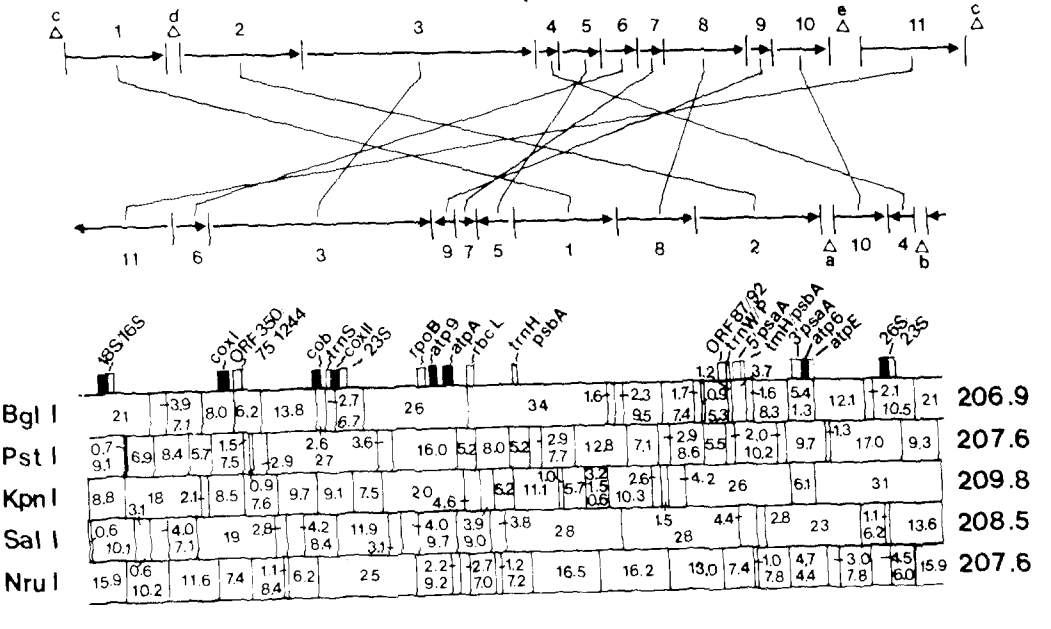
Fig. 2. Relative hybridization strengths of *B. campestris* cpDNA clones to mtDNA from six species within the family *Brassicaceae*. (*upper*) Map of the *B. campestris* chloroplast genome. PstI sites (♀) and SacI (♦) sites are indicated; the size of each cloned fragment is noted directly above the map and between enzyme recognition sites. The two uncloned regions of the genome are indicated by (*). Chloroplast genes that are present, or map close to regions present, in mtDNA are indicated above the map. (ND) denotes *B. campestris* cpDNA clones that hybridized weakly to crucifer mtDNA. However, *B. campestris* mtDNA clones containing these cpDNA-derived regions did not hybridize to tobacco cpDNA and so the identity of these shared sequences could not be determined. *Heavy horizontal lines directly below the map* indicate the position of the chloroplast large inverted repeat region. (*lower*) Schematic representation of the hybridization strengths of the cpDNA clones to the different mtDNAs. Hybridization strengths are depicted directly underneath the cpDNA fragment used as a probe. Weak hybridization between chloroplast and mitochondrial rRNA genes is not shown. Hybridizations are classified as weak (· · · ·), strong (——), or very strong (▬)



R. sativa



B. campestris



B. hirta

in kb of the cpDNA probe used. This approach to quantifying regions of DNA sequence similarity by Southern blot hybridization is less accurate in absolute terms than slot or dot blot hybridization, but has the two-fold advantage of allowing one to measure separately multiple hybridizing mtDNA regions and also to disregard cpDNA contamination of mtDNA preparations.

The mtDNAs of the three most closely related species, *B. campestris, B. hirta* and *R. sativa*, contain approximately equal amounts of cpDNA. In these species approximately 12–14 kb of DNA sequence is shared between the chloroplast and mitochondrial genomes. The mtDNA of *Crambe abyssinica* contains approximately 18 kb of cpDNA. The extra cpDNA in *Crambe abyssinica* mtDNA relative to the two *Brassica* species and *R. sativa* reflects primarily a single large *Crambe abyssinica*-specific mtDNA region that has sequence similarity to the region of the chloroplast 16S rRNA gene. *Capsella bursa-pastoris* and *A. thaliana*, the more phylogenetically distant crucifers, contain only about 5–7 kb of shared sequences in their chloroplast and mitochondrial genomes.

Location of cpDNA sequences in crucifer mtDNAs

CpDNA sequences identified in the first section of the RESULTS as being present in mtDNA were located on the known restriction maps of the mtDNAs of *B. hirta* (Palmer and Herbon 1987), *B. campestris* (Palmer and Shields 1984), and *R. sativa* (Palmer and Herbon 1986) (Fig. 3). Comparison of these maps shows that restriction enzyme sites adjacent to 11 identified cpDNA-derived regions are strictly maintained within the mtDNAs of the three species. The 11 cpDNA regions are contained within conserved blocks of mtDNA that have been rearranged within their respective mitochondrial genomes by recombination events that have occurred since the species diverged from each other (Palmer and Herbon 1987, 1988). Each of the three mtDNAs contains the same complement of shared cpDNA sequences. It appears that no significant transfer of cpDNA into the mitochondrion of these species has occurred since their divergence from a common ancestor.

Since mtDNA restriction maps are not available for *Crambe abyssinica, Capsella bursa-pastoris* and *A. thaliana* we cannot say whether their cpDNA sequences have arisen from the same transfer events that gave rise to the cpDNA regions present in the mtDNAs of the two *Brassica* species and *R. sativa*. However, we obtained data regarding this question by hybridizing *B. campestris* mtDNA clones, known to contain cpDNA sequences, to PstI digests of these mtDNAs. The mtDNA clones are large (5–10 kb) and generally contain one to several kb of *bona fide* mtDNA flanking each cpDNA-derived region. In cases where both the cpDNA probe first used to identify the interorganellar shared DNA sequences and the corresponding mtDNA clone hybridized only to the same region in each of the mtDNAs we conclude that only one transfer event has been involved. Given the large size of plant mitochondrial genomes we consider it unlikely that a cpDNA sequence would insert independently two or more times in the same place in the mitochondrial genome. These hybridization experiments (data not shown) suggest that all of the cpDNA derived regions within *Crambe abyssinica* mtDNA (except the *Crambe abyssinica*-specific 16S rDNA region) have resulted from the same transfer events that gave rise to the cpDNA sequences in the mtDNAs of the *Brassica* and *R. sativa* species. The situation for *Capsella bursa-pastoris* and *A. thaliana* is less clear and may be more complex. Some regions of shared DNA in their mitochondrial genomes (e.g. *rbc*L and the 3′ end of *psa*A, see next section) are the result of transfer events common to all six species, i.e. the cross-hybridizing *B. campestris* cpDNA and mtDNA clones hybridized to one and the same mtDNA fragment in each of the six species. However, in most cases the mtDNA clone hybridized strongly to one or more additional mtDNA fragments beyond that to which it and the cpDNA clone hybridized. This result can be interpreted in either of two ways. First, the cpDNA sequences in *A. thaliana* and/or *Capsella bursa-pastoris* mtDNA were transferred by separate events from those that gave rise to the *B. campestris* sequences. Alternatively, a single transfer event occurred, but subsequent mtDNA rearrangement has moved the cpDNA sequence to a different mtDNA location in *A. thaliana* and *Capsella bursa-pastoris* relative to the other four species.

◀ **Fig. 3.** Location of cpDNA-derived regions on the physical maps of mtDNA from *R. sativa, B. campestris* and *B. hirta*. The relative arrangement of the three mitochondrial genomes is depicted; numbers within the maps indicate restriction fragment sizes; *numbers to the right* of the maps indicate the size of each genome as determined for each enzyme. *Numbers and large arrows* between maps indicate the position and relative orientation of blocks of sequences which cross-hybridize and whose arrangement has been conserved between the genomes. *The crossing lines* connect equivalent sequence blocks. *Open boxes* indicate cpDNA-derived regions present in the mtDNAs. *Closed boxes* depict mitochondrial genes. All of the major cpDNA-derived sequences within the mtDNAs of the two *Brassica* species and *R. sativa* were present in the mitochondrial genome of a common ancestor of the three species. Mapping data are from Palmer and Herbon (1987, 1988) and Makaroff and Palmer (1987)

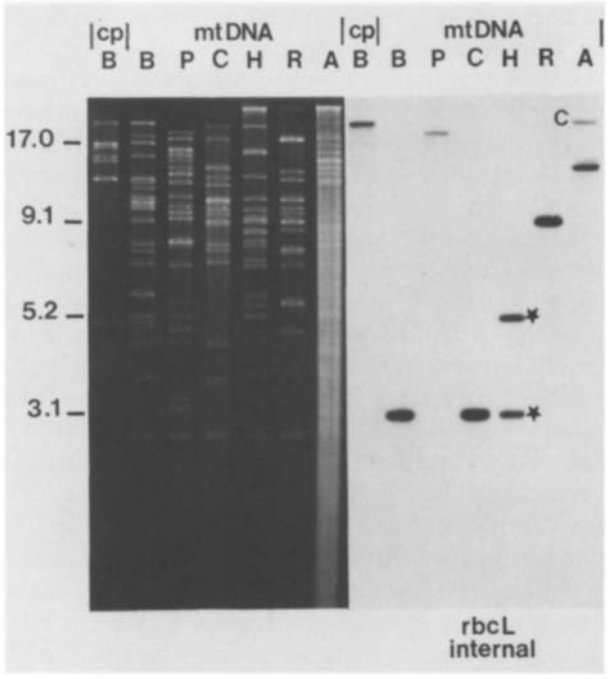


Fig. 4. Presence of the chloroplast *rbc*L sequence in the mtDNAs of all six crucifer species. mtDNAs from *B. campestris* (B), *Capsella bursa-pastoris* (P), *Crambe abyssinica* (C), *B. hirta* (H), *R. sativa* (R) and *A. thaliana* (A) and cpDNA from *B. campestris* were digested with PstI and electrophoresed on a 0.7% agarose gel. The DNAs were transferred to a Zetabind filter and probed with a clone containing a 1.1 kb PstI-HindIII fragment internal to the chloroplast *rbc*L gene. (C) denotes cpDNA contamination of the *A. thaliana* mtDNA preparation. (*) denotes restriction fragments that are representative of two different mtDNA populations within a single line of *B. hirta*. These two populations differ by a single 62 kb mtDNA inversion (see Palmer 1988)

Identification of specific cpDNA regions present in mtDNA

Regions of *B. campestris* mtDNA that hybridized to *B. campestris* cpDNA clones were used to probe Southern blots of tobacco cpDNA. The entire sequence of tobacco cpDNA has been determined (Shinozaki et al. 1986), therefore regions of the chloroplast genome that hybridize to mtDNA can be mapped and identified as to their gene content. To map DNA sequences shared between cpDNA and mtDNA as precisely as possible, tobacco cpDNA was digested with ten enzymes that cut the genome fairly frequently: AccI, BglI, ClaI, EcoRV, EcoRI, HincII, HindIII, NsiI, SspI and XbaI. Regions of *B. campestris* cpDNA that are duplicated in the *B. campestris* mitochondrial genome are shown in Fig. 2. Four of these regions map internally to identified genes encoding known chloroplast polypeptides. These genes include *rbc*L (encoding the large subunit of ribulose-1,5-bisphosphate carboxylase), *psa*A (encoding one of the two P700 chlorophyll a apoproteins of photosystem I), *rpo*B (encoding the beta subunit of chloroplast RNA polymerase), and *atp*E (encoding the epsilon subunit of coupling factor). Other regions are identified by the chloroplast gene or ORF they map closest to. Because the restriction enzymes used do not cut within these regions we cannot say whether these shared sequences map within specific genes or ORFs, or merely adjacent to them. Two mtDNA clones that had previously shown very weak hybridization to *B. campestris* cpDNA did not hybridize to tobacco cpDNA.

To confirm these results, and also to determine whether DNA sequences from these chloroplast genes are present in the mtDNAs of all six species examined, we hybridized small cpDNA probes specific to the *rbc*L, *rpo*B and *psa*A genes to Southern filters containing PstI-digested mtDNA from each species. A 1.1 kb fragment internal to the *rbc*L gene from pea (Zurawski et al. 1986) hybridized strongly to all the mtDNAs (Fig. 4). A probe for the 5′-end of *rbc*L hybridized only to *Brassica, R. sativa* and *Crambe abyssinica* mtDNA, while a 3′-end probe failed to hybridize to any of the mtDNAs (data not shown). A cloned tobacco cpDNA BamHI fragment of 1.1 kb, which is internal to the *rpo*B gene (Ohme et al. 1986), hybridized to the mtDNA of the four species in the tribe Brassiceae, but not to the mtDNAs of *Capsella bursa-pastoris* or *A. thaliana* (data not shown). A 2.4 kb BamHI clone containing the *psa*A gene from spinach (Kirsch et al. 1986) hybridized to PstI fragments of 10.2 and 9.7 kb in *Brassica, R. sativa* and *Crambe abyssinica* mtDNAs. These two mtDNA fragments are separated in *Brassica* and *R. sativa* by at least 10 kb of DNA that has no sequence similarity to the *psa*A gene (Fig. 3). A DNA clone specific for the 5′-end of *psa*A hybridized exclusively to the mtDNA 10.2 kb PstI fragment of the two *Brassica* species, *R. sativa*, and *Crambe abyssinica*, while the 3′-end of *psa*A hybridized to the 9.7 kb PstI fragment (Fig. 5). Only the 3′-end of *psa*A hybridized to mtDNAs of *Capsella bursa-pastoris* and *A. thaliana* (Fig. 5).

Discussion

We investigated the presence of cpDNA-derived sequences in the mtDNAs of six species of plants within the crucifer family in order to (1) determine if the distribution of shared DNA sequences within the chloroplast and mitochondrial genomes varies significantly over evolutionary time, and (2) estimate the evolutionary timing of interorganellar DNA transfer events.

Our results indicate that despite their apparent nonfunctionality in crucifer mtDNAs (Makaroff and Pal-

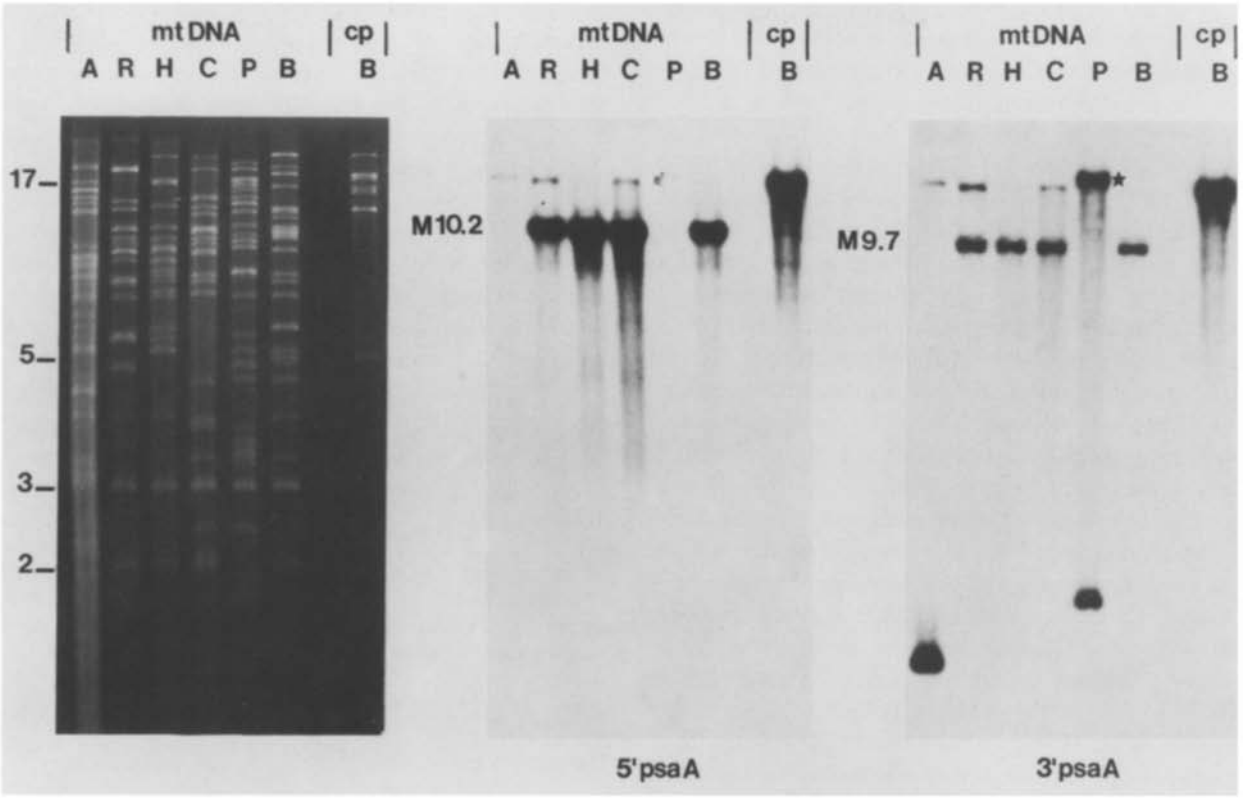


Fig. 5. Differential representation of the 5′ and 3′ ends of the chloroplast *psa*A gene in crucifer mtDNAs. Zetabind filters were prepared as described in the legend to Fig. 4 and hybridized with the indicated probes. (*) denotes an authentic *Capsella bursa-pastoris* mtDNA fragment that has sequence similarity to the 3′-end of *psa*A and does not represent cpDNA contamination of the *C. bursa-pastoris* mtDNA preparation

mer 1987), transferred cpDNA sequences can be maintained within recipient genomes over long periods of time. Chloroplast-derived sequences containing portions of the *rbc*L and *psa*A genes are present in the same locations in the mtDNAs of all six species examined and appear to have been transferred in a common ancestor of the crucifer family. Therefore, these cpDNA sequences have been stable residents of the mitochondrial genome for at least 30 million years (Cronquist 1981). The mtDNAs of the two *Brassica* species and *R. sativa* contain the same complement of cpDNA-derived sequences.

Despite the fact that 11 identified regions of chloroplast-derived sequences have been mapped on crucifer mtDNAs, the stable integration of transferred DNA by a recipient genome is a fairly infrequent event. Compared to the extent of mitochondrial genomic rearrangement that has occurred in *B. campestris, B. hirta*, and *R. sativa* (Fig. 3) since these species shared a common ancestor, the incorporation of foreign DNA by these genomes has been absolutely negligible within this same time span. The incorporation of the cpDNA sequence containing the 16S rRNA gene by *Crambe abyssinica* mtDNA is the only example of a successful transfer event that has occurred within the lineage containing *Crambe abyssinica*, the two *Brassicas*, and *R. sativa* since these species shared a common ancestor.

Only a small fraction (2–6%) of the mitochondrial genome in crucifers is cpDNA-derived. Plant mtDNAs encode significantly more genes than their animal counterparts (Makaroff and Palmer 1987), however, the increased size of plant mtDNAs is not accounted for by an increased coding capacity. These observations, combined with the fact that plant mitochondrial genomes vary over an enormous size range (215–2,400 kb) (Lebacq and Vedel 1981; Palmer et al. 1983a; Ward et al. 1981), suggest that much of the plant mtDNA may be "junk" DNA which is potentially nuclear in origin. Although experiments to test for the origin of extraneous mtDNA in plants have not yet been carried out, a recent report (Schuster and Brennicke 1987) has demonstrated the presence of a 528 bp sequence in the mitochondrial genome of *Oenothera* which has sequence similarity to the nuclear encoded small rRNA gene. Whether more of the mitochondrial genome is nuclear derived remains to be determined.

A cpDNA region containing the 16S rRNA gene is found in *Crambe abyssinica* mtDNA and is not present

in any of the other mtDNAs examined. Portions of the chloroplast *psa*A and *rpo*B genes are present only in the mtDNA of the four more closely related crucifers while other cpDNA derived sequences (3′-*psa*A, internal region of *rbc*L) are common to the mtDNAs of all six species. These results are highly suggestive of a serial transfer of cpDNA into the mitochondrial genome over evolutionary time (Fig. 1). Alternatively, selective loss of cpDNA from the mitochondrial genome as a result of genomic rearrangements and deletions could account for the variation in the amount of cpDNA/mtDNA shared sequences within these species. It appears that the 5′-end of the *rbc*L sequence has been lost from *Capsella bursa-pastoris* and *A. thaliana* mtDNA. However, in general, we feel that the latter explanation is less likely considering the fact that cpDNA-derived sequences have been highly conserved within the mitochondrial genomes of the two *Brassica* species and *R. sativa* despite extensive mtDNA rearrangement (Fig. 3).

Tobacco and *Marchantia* cpDNAs contain six open reading frames that have sequence similarity to genes encoding components of the human mitochondrial NADH dehydrogenase complex (Ohyama et al. 1986; Shinozaki et al. 1986). Four of these ORFs map within the small single-copy region of the tobacco chloroplast genome. None of our clones from the small single copy-region of *B. campestris* cpDNA, which is coextensive with the tobacco small single copy region (Palmer et al. 1983b), hybridized to any crucifer mtDNA. The absence of detectable cpDNA/mtDNA sequence similarities involving *ndh* genes, together with the widespread presence (Ohyama et al. 1986; Shinozaki et al. 1986; Meng et al. 1986) and active expression of the chloroplast *ndh* genes (Matsubayashi et al. 1987), suggests that the two sets of organelle *ndh* genes have had a very long period of separate evolutionary history, probably dating back to the separate endosymbiotic origins of the two organelles.

Despite the well documented existence of promiscuous DNA, the mechanism or mechanisms by which intracellular DNA promiscuity arises remain as yet only speculative. Does the transfer process involve direct physical contact between cellular organelles and subsequent intermolecular recombination, the direct uptake of sequences released into the cytoplasm from broken or lysed organelles, or vector-mediated DNA transfer? A second central question concerns the chemical nature of the nucleic acid intermediate involved in the transfer process. Is it DNA, RNA or both? Within the 12 kb region shared between the maize chloroplast and mitochondrial genome (Stern and Lonsdale 1982), the presence of two oppositely transcribed sets of genes, the tRNA-rRNA operon (Strittmatter and Kössel 1984; Dormann-Przybyl et al. 1986) and the *rps*7/*rps*12 operon (Giese et al. 1987) (including spacer sequences and several introns), suggests that the transfer of this sequence involved a DNA rather than an RNA intermediate. However, the recent discovery of an open reading frame in the mitochondrial genome of *Oenothera* with sequence similarity to reverse transcriptase (Schuster and Brennicke 1987) has raised the possibility that some interorganellar DNA transfer may occur via RNA, followed by local reverse transcription and genome integration (Schuster and Brennicke 1987). The identification of a cpDNA sequence that has recently entered the mitochondrial genome (16S rRNA gene in *Crambe abyssinica*) may provide an excellent opportunity to discover sequence clues regarding the mechanism of transfer of cpDNA into the mitochondrial genome.

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