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Molecular systematics of *Brassica* and allied genera (Subtribe Brassicinae, Brassiceae) – chloroplast genome and cytodeme congruence

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Summary. Chloroplast DNA restriction sites for 20 endonucleases were mapped using cpDNA probes from *Brassica juncea* and site variation was surveyed in 33 diploid taxa of the Subtribe Brassicinae. A total of 419 mutations was observed, including both site (i.e., gain/loss) and fragment length (i.e., insertions or deletions); 221 (53%) mutations showed variation at the interspecific level. Phylogenetic analysis indicated a clear division of the subtribe into two ancient evolutionary lineages. These were (I) the "Nigra" lineage: *Brassica nigra*, *B. fruticulosa*, *B. tournefortii*, *Sinapis pubescens*, *S. alba*, *S. flexuosa*, *S. arvensis*, *Coincya cheiranthos*, *Erucastrum canariense*, and *Hirschfeldia incana*, and (II) the "Rapa/Oleracea" lineage: *Brassica rapa*, *B. oleracea* ssp. *oleracea* and ssp. *alboglabra*, *B. rupestris-villosa* complex (*B. rupestris*, *B. drepanensis*, *B. macrocarpa*, *B. villosa*), *B. barrelieri*, *B. deflexa*, *B. oxyrrhina*, *B. gravinae*, *Diplotaxis erucoides*, *D. tenuifolia*, *Eruca sativa*, *Raphanus raphanistrum*, *R. sativus*, and *Sinapis aucheri*. In the "Nigra" lineage, *Brassica nigra* was most closely related to the annual *Sinapis* species, *S. arvensis* and *S. alba*. In the "Rapa/Oleracea" lineage, the *Brassica rapa* and *B. oleracea* genomes formed a distinct group whose closest relatives were the wild species of the *B. oleracea* (n = 9) complex (i.e., *B. rupestris-villosa* complex). Species with n = 7 chromosomes exist in both lineages. *Hirschfeldia incana* (n = 7), in the "Nigra" lineage, was most closely related to *Sinapis pubescens*. In the "Rapa/Oleracea" lineage three taxa with n = 7 – *B. deflexa*, *D. erucoides*, and *S. aucheri* – were closely related, advanced in the lineage, and were the closest apparent relatives (particularly *D. erucoides*) to *B. rapa*, *B. oleracea*, and its wild relatives. Levels of genetic divergence suggested by the cpDNA data were consistent with cytodeme recognition in the subtribe, but provided evidence for inconsistencies in the current generic delimitations based on morphology. Very low levels of genetic divergence were evident among taxa/accessions within a cytodeme. *Raphanus* was closely related to the *Brassica rapa* and *B. oleracea* genomes and clearly belongs in Subtribe Brassicinae. Several cytoplasmic genetic markers of potential use in plant breeding programs were identified for each of the cytodemes.

Key words: *Brassica* – Subtribe Brassicinae – Chloroplast DNA – Restriction site variation – Molecular systematics

Introduction

The importance of both chloroplast DNA (cpDNA) (Palmer et al. 1983; Erickson et al. 1983; Yanagino et al. 1987) and nuclear DNA (Song et al. 1988a, b, 1990; Hosaka et al. 1990) variation in resolving species relationships in *Brassica* and related genera has been demonstrated. Inferred phylogenies were similar, indicating that the evolution of the maternally inherited chloroplast genome is highly correlated with that of the biparentally inherited nuclear genome. In contrast, evolutionary relationships based on morphometric data (e.g., Takahata and Hinata 1986) were not always congruent with cytological and molecular data.

The present paper compares restriction site variation in the chloroplast genome of several species of *Brassica* and allied genera of the Subtribe Brassicinae, including *Coincya*, *Diplotaxis*, *Eruca*, *Erucastrum*, *Hirschfeldia*, *Raphanus*, and *Sinapis* (Table 1). Based primarily on morphological data, Schulz (1919) recognized 11 genera and about 90 species in the Subtribe Brassicinae, one of six subtribes in the tribe Brassiceae (Schulz 1919; Gómez-

Table 1. Taxa, chromosome number (n), and source of seed for plant material included in this study. DNAs were obtained from single plants of each accession

Taxa	Code	n[a]	Source[b]
Brassica			
B. barrelieri (L.) Janka	BB	10	PGRC No. 13218 (BCN 3232)
B. deflexa Boiss.	BD	7	GCC No. 3713-75 (BCN 3210)
B. fruticulosa Cyr.	BF	8	BGP (BCN 3020)
B. gravinae Ten.	BG	10	BGL No. 387 (BCN 3179)
B. nigra (L.) Koch	BN	8	BGP (BCN 3023)
B. oleracea L.	BO	9	
ssp. *oleracea*			
– Wild accession	BO1	9	CD No. 989 (BCN 3085)
– Crop accession – Broccoli	BO2	9	FIN (BCN 3489)
ssp. *alboglabra* L. H. Bailey	BA	9	PGRC No. 7784 (BCN 3017)
B. oxyrrhina Coss.	BX	9	BIC (BCN 3141)
B. rapa L.	BC	10	
– Weedy accession, cornfield	BC1	10	Notre-Dame-de-Lourdes, Canada (BCN 2984)
– Oriental crop accession	BC2	10	Japanese Greens cv "Shirona" (BCN 3525)
B. rupestris-villosa complex	BRV	9	
B. rupestris Rafin.	BR	9	BGP (BCN 3025)
B. drepanensis (Car.) Dam	BP	9	BGP (BCN 3019)
B. macrocarpa Guss.	BM	9	BGP (BCN 3022)
B. villosa Biv.	BV	9	BGP (BCN 3026)
B. tournefortii Gouan.	BT	10	WGB No. 4531 (BCN 3439)
Coincya cheiranthos (Vill.) Greut. & Burd.	CC	12	BIC (BCN 3141)
Diplotaxis			
D. erucoides (L.) DC	DE	7	BGS No. 330 (BCN 3463)
D. tenuifolia (L.) DC	DT	11	BGPa No. 154 (BCN 3416)
Eruca sativa Mill.	ES	11	BGN No. 24 (BCN 3474)
Erucastrum canariense Webb & Berth	EC	9	CD No. 1065 (BCN 3091)
Hirschfeldia incana (L.) Lagr.	III	7	Djebel Thaya, Algeria (BCN 2700)
Raphanus	RRS	9	
R. raphanistrum L.	RR	9	St.-Victoire-de-Sorel, Canada (BCN 3449)
R. sativus L.	RS	9	ZBG No. 181 (BCN 3520)
Sinapis			
S. alba L.	SA	12	
ssp. *alba* L.	SA1	12	PGRC No. 4671-"Sabre" (BCN 3230)
ssp. *mairei* (H. Lindb.) Maire	SA2	12	GAT No. SIN20/81 (BCN 2962)
S. flexuosa Poir.	SF	12	BGB No. 7-24 (BCN 3225)
S. arvensis L.	SR	9	
ssp. *arvensis* L.	SR1	9	Hamman Zeid, Algeria (BCN 2707)
ssp. *nilotica* O. E. Schulz	SR2	9	ZBG No. 185 (BCN 3521)
S. aucheri (Boiss.) O. E. Schulz	SO	7	GCC No. 3735-75 (BCN 3211)
S. pubescens s. lat.	SP	9, 18	
ssp. *pubescens* L.	SP1	9	Setif, Algeria (BCN 2713)
ssp. *virgata* (Battand.) Baill.	SP2	9	Cap-Carbon, Algeria (BCN 2691)
ssp. *aristidis* (Pomel.) Maire	SP3	9	Berlin, DGR (BCN 2928)
ssp. *indurata* (Coss.) Battand	SP4	9	Hamman Zeid, Algeria (BCN 2705)
S. boivinii Baill.	SP5	18	Chabet el Akra, Algeria (BCN 2695)
Outgroup			
Reboudia microcarpa (Boiss.) Coss. & Dur.	RE	8	CD No. 1138 (BCN 3093)
Sisymbrium loeselii L.	OT	–	ZBG No. 172 (BCN 3519)

[a] Chromosome numbers are from Gómez-Campo and Hinata (1980) and Baillargeon (1986)

[b] BGB = Botanic Garden, Barcelona, Spain; BGL = Botanic Garden, University of London, Surrey, UK, No. 387; BGP = Botanic Garden, University of Palermo, Italy; BGPa = Botanical Garden, Pavia University, Italy; BGN = Botanical Garden, Neubauer Pedagogic High School, Mühlhausen, Germany; BGS = Botanical Garden, Salzburg University, Austria; BIC = Botanical Institute, Coimbra University, Portugal; CD = Copenhagen, Denmark; FIN = Fines Seed Co., Ottawa, Canada; GAT = Genetic and Breeding Institute, Academy of Sciences, Gatersleben, Germany; GCC = Gómez-Campo Collection, E.T.S.I.A., Madrid, Spain; PGRC = Plant Gene Resources, Ottawa, Canada; WGB = Wellsbourne Gene Bank, UK; and ZBG = Zahrada Botanic Garden, Tabor, Czechoslovakia. Note BCN: collection number on herbarium labels for specimens deposited at Herbarium, Agriculture Canada, Ottawa

Campo 1980). This was later revised to nine genera and about 100 species, by excluding the genus *Reboudia* and merging *Brassicella* into *Hutera* (=*Coincya*) (Gómez-Campo 1980). However, because traditional, morphologically based taxonomies are difficult, subtribe and generic boundaries in the Brassiceae may not reflect natural groups (Gómez-Campo 1980; Al-Shehbaz 1984). Species relationships in the subtribe have been studied cytogenetically (reviewed in Prakash and Hinata 1980; Takahata and Hinata 1983), chemically, and morphologically (reviewed in Takahata and Hinata 1986). On the basis of chromosome number and crossing ability, Harberd (1976) defined the *Brassica* "coenospecies" as the group sufficiently related to the six cultivated species of *Brassica* to be capable of experimental hybridization with them. It has been classified into 42 diploid and 11 tetraploid cytodemes or crossing groups (Harberd 1976; Takahata and Hinata 1983), and corresponds closely to the taxonomic Subtribe Brassicinae, with the inclusion of *Raphanus* and *Enarthrocarpus*. Chloroplast DNA studies have also suggested that *Raphanus* belongs in the Brassicinae (Palmer et al. 1983). Disparities between morphologically based generic delimitations and genetic relatedness are reported in the subtribe, where homology among *Brassica* and allied genera was often higher than between different genomes of *Brassica* (Mizushima 1980). A more natural classification in the subtribe would be achieved by grouping closely related species and genera and working upward; Takahata and Hinata (1983) suggested grouping species on the basis of cytodemes, since these reflected the sexual isolation of genomes.

The first objective of the present study was to prepare restriction site maps of the entire chloroplast genome for 20 endonucleases, using cpDNA probes from *Brassica juncea*. Secondly, the data, based on ca. 800 restriction sites, will be used to test taxonomic classifications and species and cytodeme relationships among genera and within selected genera (*Brassica* and *Sinapis*), and to assess the correlation between cpDNA data, cytodeme status, chromosome number, and primitiveness in the subtribe.

Materials and methods

Plant material

The sources of seeds and chromosome number for each of the taxa included in the study are given in Table 1. From three to six plants were grown from seeds of each accession in a greenhouse. Verified voucher specimens of mature plants of each accession were deposited in the Vascular Plant Herbarium, Biosystematics Research Centre, Agriculture Canada, Ottawa. DNAs were obtained from single plants in each accession.

Molecular methods

Total cellular DNA from each individual plant was isolated from 3–4 g of leaf material collected and stored at −80 °C. Total DNA was extracted using a modified CTAB method (Doyle and Doyle 1987), including extraction with phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v), and chloroform/isoamyl alcohol (24:1, v/v) precipitation with sodium acetate (pH 5.5) and ethanol at −80 °C, as well as purification by CsCl/ethidium bromide density gradient centrifugation. Molecular methods presented below are modifications of those described in Sambrook et al. (1989). The DNA was digested with each of 20 restriction endonucleases (listed in Fig. 1 and Appendix). Restriction fragments were separated by electrophoresis in 0.8% agarose gels in 89 m*M* TRIS, 2.5m*M* EDTA, and 89 m*M* boric acid. After an 8-min treatment in 0.25 *M* HCl and denaturation in 0.4 *M* NaOH, the DNA was transferred to Biotrans nylon membrane (ICN Biomedicals, Canada) using bidirectional dry Southern blotting procedures, which resulted in duplicate filters of each gel. DNA was immobilized by exposing filters to short UV radiation for 3 min and drying at 80 °C for 2 h under vacuum. Filters were sequentially probed with 25 clones representing the entire chloroplast genome of *B. juncea*, with the exception of two small regions, i.e., a 7.0-kb fragment between clones 8 and 10 and a 1.1-kb fragment between clones 16 and 19 (Fig. 1). Clones were provided by J. Palmer, University of Indiana, and five of the original clones were subcloned in order to facilitate mapping. Clone number and size are shown in Fig. 1. Probe DNA was labelled by Nick-translation with deoxycytidine 5′-(^{32}P) triphosphate (6,000 Ci mmol^{-1}) from Dupont, Canada, and filters were prehybridized in 0.5% nonfat dry milk, 2% SDS, and 4 × SSC and hybridized at 65 °C. Autoradiography was done with XAR-2 film. Membrane filters were stripped of radioactive probe by repeated rinses with a boiling 0.1 × SSC solution and were then reprobed successively.

Data analysis

Restriction site maps for each of the endonucleases surveyed, relative to clones of the *B. juncea* chloroplast genome, were produced (Fig. 1 A, B) by sizing all fragments for each clone and sequentially comparing adjacent clones. In the absence of site polymorphisms, the relative position of small fragments within large clones was not always possible. All sites were numbered and the site mutations (Appendix) were summarized as gain or loss. In addition, several fragment length mutations were recorded. Insertional/deletional mutations were confirmed by the detection of the same length variation for other enzymes in that particular clone; each mutation was recorded once for a single enzyme (Appendix) and indicated in Fig. 1 for the other enzymes (details also available from authors on request). Other length mutations listed in the Appendix likely represent site gains or losses involving small unresolved fragments.

The data (Appendix) were subjected to both phenetic clustering and phylogenetic analyses. Each of the 419 restriction site or length mutations was treated as a two-state variable. Taxa were clustered using a simple matching similarity coefficient and the average linkage (unweighted paired group) method, employing the computer program "Numerical Taxonomy and Multivariate Analysis System" (NTSYS-pc) version 1.40 developed by F. J. Rohlf (State University of New York, Stony Brook).

A phylogenetic analysis of species relationships was conducted using the computer program "Phylogenetic Analysis Using Wagner Parsimony" (PAUP) version 2.4 developed by D. L. Swofford (Illinois Natural History Survey; Options Hold=1, Swap=Alternate, Addseq=Closest, and Mulpars). The shortest phylogenetic tree(s) were calculated on the basis of all mutations in the Appendix that were shared by two or more taxa. The number of mutations unique to a single species is indicated in brackets on the terminal branch points. Alternative methods to root the tree were examined, including outgroup, midpoint, and

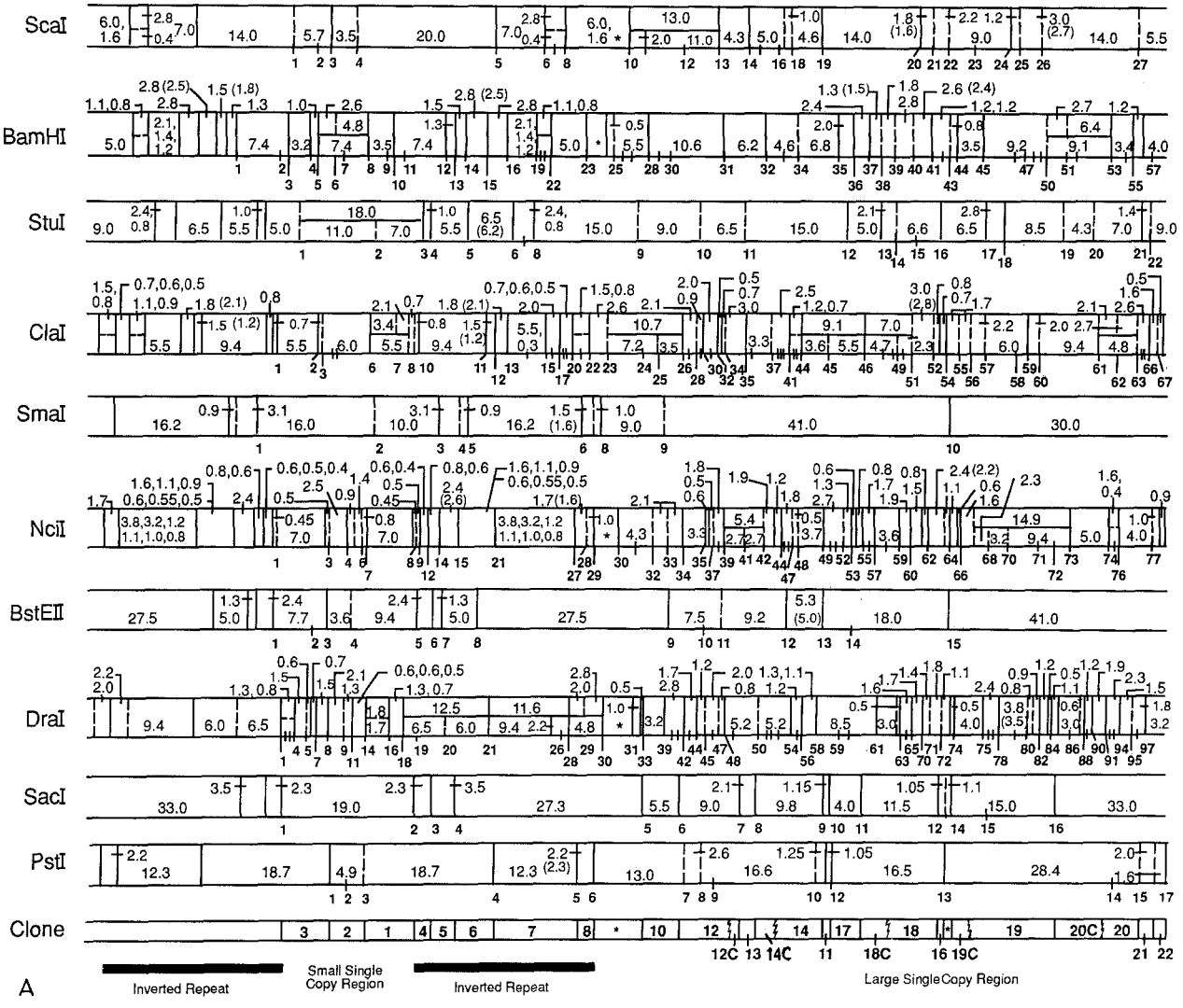
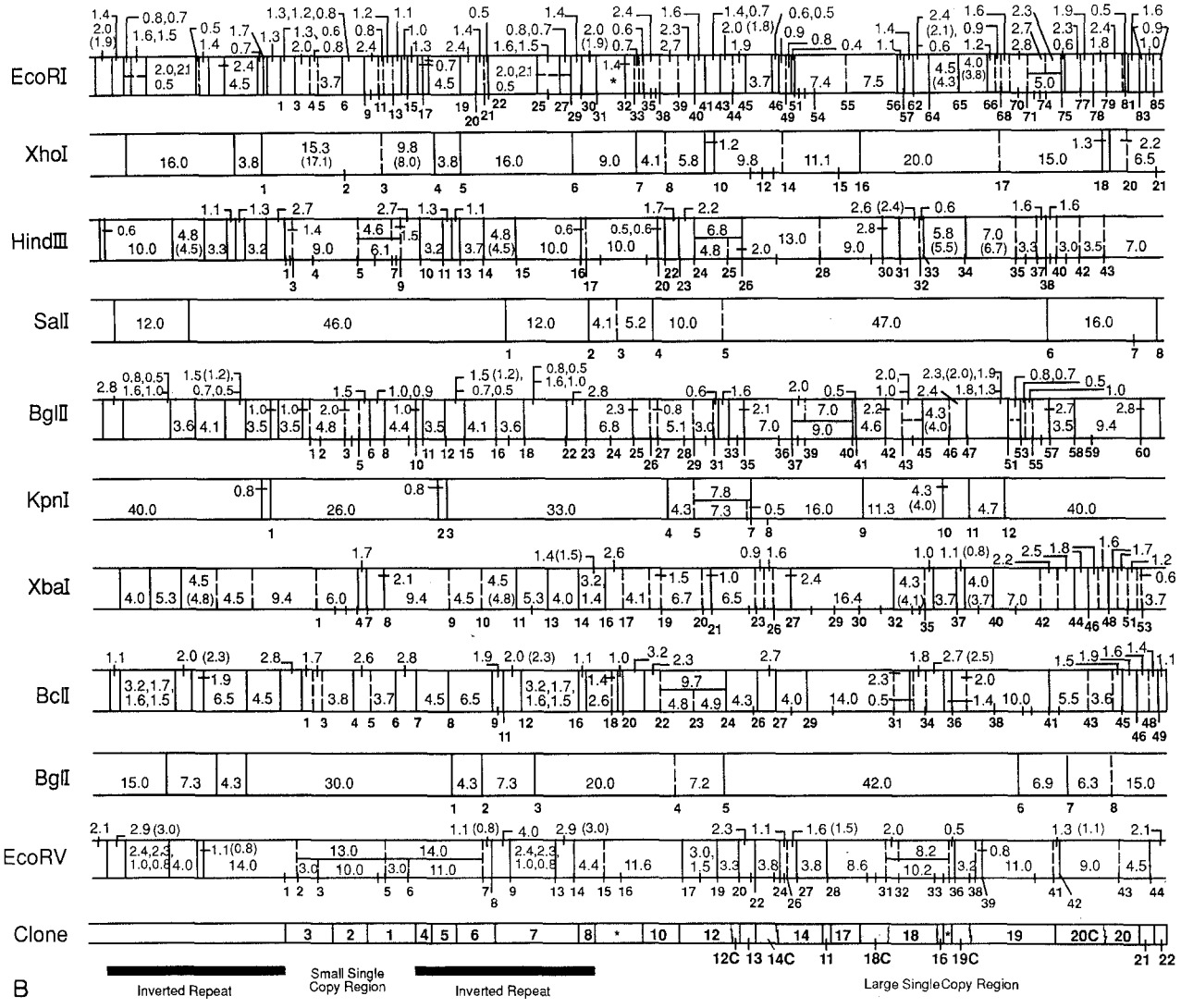


Fig. 1 A and B. Chloroplast DNA restriction maps of 20 restriction endonucleases relative to heterologous chloroplast DNA clones used in this study. Clones are arranged in a linear order; the inverted repeats are indicated by *black bars*, which separate the large and small single-copy regions of the genome. Clones were obtained from *Brassica juncea* PstI or SacI fragments (J. D. Palmer, unpublished results). Size (kb) of each clone was: clone 1 (7.1), 2 (4.9), 3 (7.0), 4 (2.3), 5 (3.5), 6 (5.8), 7 (12.3), 8 (2.2), 10 (5.5), 11 (1.15), 12 (9.0), 13 (2.1), 14 (9.8), 16 (1.05), 17 (4.0), 18 (11.5), 19 (15.0), 20 (12.3), 21 (2.0), and 22 (1.6). Subclones of above clones, indicated by a *jagged line*, include: 12C (1.5 kb SacI/BamHI), 14C (2.2 kb SacI/PvuII), 18C (4.0 kb SacI/PvuII), 19C (2.5 kb SacI/HindIII), and 20C (7.3 kb SacI/BamHI). The *asterisks* (*) indicate regions of the genome that have not been cloned, i.e., a 7.0-kb fragment between clones 8 and 10 and a 1.1-kb fragment between clones 16 and 19. For each enzyme, all mapped sites are numbered from *left* to *right*. All invariant sites are mapped; these are indicated by either a *solid line* or a *comma* if the site could not be precisely mapped. Variable or polymorphic sites are indicated by either a *dashed line* for mutations present in two or more species, or simply by a number for mutations in a single species, with details described in the Appendix. *Asterisks* indicate regions that were not mapped and correspond to the areas of the genome that were not cloned. Length mutations for individual fragments are indicated with the alternative length shown in brackets. Note: not all possible fragment lengths could be mapped for every taxa – see Appendix for specific genomic markers

Lundberg rooting. The two outgroup taxa included: *Sisymbrium loeselii* (Tribe Sisymbrieae, Hedge 1976) and *Reboudia microcarpa*, originally placed in the Brassicinae, with its current position in Tribe Brassiceae unclear (Gómez-Campo 1980). All three procedures yielded the same two lineages; results are shown using midpoint rooting, including *Reboudia*. Ten equally parsimonious trees were obtained (available from the authors on request); these showed similar topologies both in terms of species composition within each of the two lineages and in the relative grouping of species within each lineage. A composite tree is represented in Fig. 2.

Results and discussion

The 793 restriction sites mapped (Fig. 1) represented 4,758 nucleotide bases or 3.2% of the chloroplast ge-



nome. In general, very low levels of intraspecific variation in chloroplast DNA occur in plants (Palmer 1987), and in the present study no variation was found among plants in an accession and very low levels of variation, i.e., 0–0.01%, were found within a given species. In the taxa surveyed, 419 mutations, including both site and length mutations, were observed (Appendix), with 221 (53%) of these showing variation at the interspecific level.

Both phylogenetic analyses (Fig. 2) and phenetic clustering (results available from the authors on request) of the cpDNA data indicated a clear division of the subtribe into two separate evolutionary lineages. The first, hereafter referred to as the "Nigra" lineage, included: *Brassica nigra*, *B. fruticulosa*, *B. tournefortii*, *Sinapis pubescens* s. lat., *S. alba*, *S. flexuosa*, *S. arvensis*, *Hirschfeldia incana*, *Erucastrum canariense*, and *Coincya cheiranthos*. The second lineage, hereafter referred to as the "Rapa/Oleracea" lineage, included: *Brassica rapa*, *B. oleracea*, *B. rupestris-villosa* complex (*B. rupestris*, *B. drepanensis*, *B. macrocarpa*, *B. villosa*), *B. barrelieri*, *B. deflexa*, *B. oxyrrhina*, *B. gravinae*, *Diplotaxis erucoides*, *D. tenuifolia*, *Eruca sativa*, *Raphanus raphanistrum*, *R. sativus*, and *Sinapis aucheri*. A high level of congruence was observed between recognized cytodemes or crossing groups in the subtribe and the clusters defined by the cpDNA data.

The separation of cultivated *Brassica*s into two lineages (*B. nigra* versus *B. rapa* and *B. oleracea*) had been suggested from earlier cpDNA data (Palmer et al. 1983; Erickson et al. 1983; Yanagino et al. 1987) and nuclear RFLP data (Song et al. 1988a, b, 1990). Other data, including chromosome pairing, isozymes, and morphometric data (reviewed in Prakash and Hinata 1980; Song et al. 1988a), also indicated that *B. nigra* is more distant from *B. oleracea* and *B. rapa* than the latter two species are from each other. Our data suggest that the two lineages diverged very early in the evolution of the Brassicinae, and prior to the evolution of distinct cytodemes in

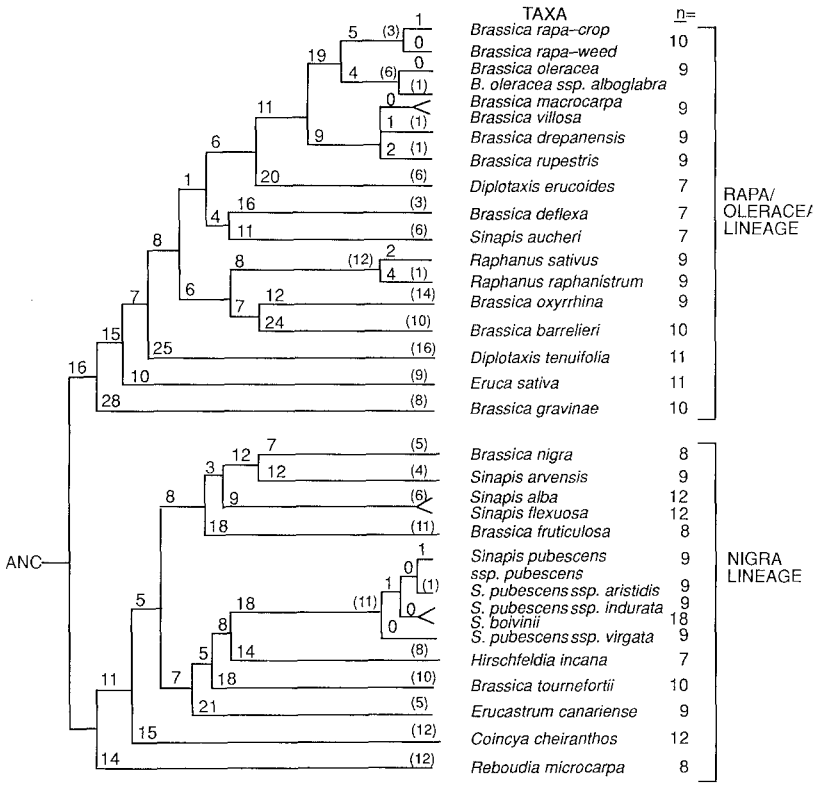


Fig. 2. Selected phylogenetic tree for the Subtribe Brassicinae based on PAUP analyses of the chloroplast DNA restriction site/length mutations in the Appendix, which are shared by two or more taxa/accessions. Tree length is 489 steps, consistency index, 0.491. Tree topology indicates how accessions are related, and branch length (numbers above the branches) indicates the minimal number of mutational steps occurring during the evolution of a particular taxa. Mutations unique to a given species and to the genus *Raphanus* (number indicated in brackets at end of branch) should be added to determine terminal branch length. ANC shows the common hypothetical common ancestor

the subtribe. Our cpDNA data does not support the proposal by Mizushima (1980) and Prakash and Hinata (1980) that the three basic diploid species of *Brassica* are aneuploids, evolving in ascending order from a common ancestor with a basic chromosome number of n = 6 (7). Both lineages contained species with n = 7 chromosomes, i.e., *Hirschfeldia incana* in the Nigra lineage and *Brassica deflexa*, *Diplotaxis erucoides*, and *Sinapis aucheri* in the Rapa/Oleracea lineage, suggesting separate evolutionary pathways and the absence of n = 7 species at the basal position in the tree.

Parallel evolution of several cpDNA mutations (both reversal and convergent parallelisms) appears to have occurred independently in the separate lineages. These affect the consistency index of the tree in Fig. 2 (=0.49) such that when each lineage was analyzed separately, markedly increased consistency indices were obtained (=0.70) (results available from authors on request).

Rapa/Oleracea lineage

Our cpDNA data agrees with nuclear genomic data (Song et al. 1988 b, 1990; Hosaka et al. 1990) in confirming the close relationship of *Brassica rapa* (n = 10) and *B. oleracea* (n = 9) genomes. Within each species, single mutational differences were detected between the crop and weedy accessions of *B. rapa* and between the crop and wild accessions of *B. oleracea* ssp. *oleracea* and ssp. *alboglabra*. Our cpDNA data and nuclear DNA studies (Song et al. 1988 b) support the taxonomic treatment of *B. alboglabra* as a subspecies of *B. oleracea* (Snogerup et al. 1990). The closest sister group to the rapa/oleracea genome was the wild species of the *B. rupestris-villosa* (n = 9) complex, which formed a distinct subgroup differing within by only four mutations. All taxa in this complex are interfertile with *B. oleracea* and are members of the *B. oleracea* cytodeme (Harberd 1976). The four are similar morphologically, distinguished primarily on degree of tomentum of the leaves, and in a recent treatment, Snogerup et al. (1990) included *B. drepanensis* within *B. villosa*. *Brassica villosa*, *B. drepanensis*, and *B. macrocarpa* are restricted in distribution to Sicily, whereas *B. rupestris* occurs in Sicily and southern Italy (Snogerup et al. 1990). The very large, characteristic fruits of *B. macrocarpa* had previously caused taxonomists to doubt its placement in the genus *Brassica*; however, growth habit, chromosome number (Snogerup et al. 1990), nuclear DNA data (Hosaka et al. 1990; Song et al. 1990), and our cpDNA data clearly place it within the *B. rupestris-villosa* complex. The low levels of cpDNA variation and morphological divergence among taxa within this complex are more consistent with subspecific recognition rather than species rank.

Brassica deflexa, *Diplotaxis erucoides*, and *Sinapis aucheri*, all n = 7, were closely related to each other, advanced in the lineage, and were the closest apparent relatives (particularly *D. erucoides*), to *B. rapa*, *B. oleracea*, and its wild relatives. In contrast, there is no apparent

correlation with morphological characters and the chromosome number n = 7 in the subtribe. In numerical taxonomic studies of morphological traits, Takahata and Hinata (1986) found that of the n = 7 species studied (*Brassica deflexa*, *Diplotaxis erucoides*, *Erucastrum varium*, *Sinapis aucheri*, and *Hirschfeldia incana*) each represented a distinct morphological group. The genetic closeness of *D. erucoides* to *B. oleracea* had been indicated by hybrid formation and meiotic analyses (Mizushima 1980). In contrast, Quiros et al. (1988), crossed *Hirschfeldia incana* and *Diplotaxis erucoides* with *B. nigra* and *B. oleracea* and found that all hybrid combinations demonstrated low fertility, which indicated a strong divergence of the four genomes.

Brassica oxyrrhina, treated as a subspecies of *B. barrelieri* in Flora Europea (Tutin et al. 1964), is recognized as a separate cytodeme (Harberd 1976; Takahata and Hinata 1983). Our cpDNA data support species rank and separate specific cytodeme status of each taxa. The distinct nuclear genome of *B. oxyrrhina* from other n = 9 species of *Brassica* was also indicated in the studies of Hosaka et al. (1990). Similarly, *Brassica gravinae*, recognized as a separate cytodeme (Takahata and Hinata 1983), has a distinct chloroplast genome, which occupies a basal position in the lineage.

Raphanus spp., *Eruca sativa*, and *Diplotaxis tenuifolia* also occurred in this lineage. Morphologically, *Raphanus* has been placed in either Subtribe Raphaninae or in an intermediate position with subtribe Brassicinae (Gómez-Campo 1980). Data presented here, crossing data (Harberd 1976), and other molecular data (Palmer et al. 1983; Yanagino et al. 1987; Song et al. 1990) confirm the placement of this genus in the Brassicinae. The weedy species *R. raphanistrum* and the crop species *R. sativus*, which belong to the same cytodeme, differed by only four mutations. The close relationship of *Eruca sativa* (genome E) to *B. rapa* and *B. oleracea* was evident from meiotic analyses (Mizushima 1980; Prakash and Hinata 1980).

Nigra lineage

Brassica nigra was most closely related to *Sinapis arvensis*, which, together with *S. alba* and *S. flexuosa*, formed a distinct cpDNA subgroup. The close relationship between *S. arvensis* and *B. nigra* was also strongly suggested by other data sets, i.e., cytological (Prakash and Hinata 1980), isozyme (Coulthart and Denford 1982), and nuclear DNA (Song et al. 1988a). *Sinapis alba* L. and *S. flexuosa* Poiret (both n = 12) were considered separate cytodemes by Harberd (1976), but no divergence in their cpDNA genomes was detected. The cpDNA data also indicated the close genetic relationship of *Sinapis pubescens* to *Hirschfeldia incana*. This closeness is also evident morphologically, where a single trait, degree of sepal erectness, is used to separate them (Schulz 1919; Tutin et al. 1964); it is also reflected in prior taxonomic treatments of *H. incana* = *S. incana* L. Within *S. pubescens* s. lat., there were only three mutational differences, supporting subspecific ranking for the five taxa recognized in this complex. The polyploid *S. boivinii*, described by Baillargeon (1986), did not differ from ssp. *indurata*, confirming the inclusion of this taxa in the *pubescens* complex.

Two other *Brassica* taxa formed part of this lineage: *B. tournefortii* and *B. fruticulosa*, both of which are distinct cytodemes (Harberd 1976). The cpDNA studies of Yanagino et al. (1987) also indicated that these two species were more closely related to *B. nigra* than to *B. rapa* and *B. oleracea*. The single representative taxa of the genera *Coincya* and *Erucastrum* were included in this lineage.

The genus *Reboudia* is aligned at the base of the more primitive Nigra lineage, lending support for Schulz's (1919) inclusion of the genus *Reboudia* in the subtribe. Harberd (1976) indicated, however, that the *Reboudia* cytodeme should be excluded from the *Brassica* coenospecies. Further studies of other subtribes in the Brassiceae are required to confirm the position of this genus.

Taxonomic classification

Taxonomy of Sinapis. Within *Sinapis*, the four cpDNA groups observed correspond to the four sections recognized by Schulz (1936) and Baillargeon (1986). These included: the perennial Section *Eriosinapis* (*S. pubescens*), the annual Section *Sinapis* (*S. alba* and *S. flexuosa*), the annual Section *Ceratosinapis* (*S. arvensis*), and the annual Section *Chondrosinapis* (*S. aucheri*). However, our data indicated that the genus is not monophyletic, confirming the anomalous status of *S. aucheri*. This had been suggested earlier on the basis of its distinct fruit morphology, i.e., long, torulose, corky, six- to ten-seeded beaks, unique chromosome number (n = 7), and endemism to Western Iran and eastern Iraq, as compared with the Mediterranean distribution for the rest of *Sinapis* (Al-Shehbaz 1985; Schulz 1936). Our data suggest that *S. aucheri* is quite close to the genus *Raphanus* (only Section *Raphanus* was available for this study). The nomenclature and taxonomy of *S. aucheri* are often confused with a second section of *Raphanus*, *Hesperidopsis* (Al-Shehbaz 1985; Baillargeon 1985, 1986). The latter contains a narrow endemic, "*R. boissieri* Al-Shehbaz" [nom. illeg., = *Quidproquo confusum* Greuter & Burdet., according to Baillargeon (1985)], which is also endemic to Iran and Iraq; the suggested relationship between these taxa merits further investigation. The data supported the monotypic treatment of *Eriosinapis*, as *S. pubescens* L. (Schulz 1936), rather than the species rank assigned to these subspecies (Baillargeon 1986). Within Section

Sinapis, no differences were observed in the cpDNA genomes of *S. alba* and *S. flexuosa*, suggesting a recent divergence and reproductive isolation of these two species. Geographically overlapping, *S. flexuosa* is restricted to southern Spain, Morocco, and Algeria, compared to the widespread European and Mediterranean distribution of *S. alba* (Baillargeon 1986). The molecular data does not support the recent morphological analysis of the genus *Sinapis* by Takahata and Hinata (1986), which split the genus into two, including *S. arvensis* in one group and *S. pubescens*, *S. alba*, and *S. aucheri* into another group.

Taxonomy of Subtribe Brassicinae. The evidence for two distinct lineages in the subtribe indicates polyphyletic origins for at least two of the genera studied to date, *Brassica* and *Sinapis*. The taxonomic confusion between *Sinapis* and *Brassica* is historic (reviewed in Baillargeon 1986), originating with the selection of *Sinapis nigra* L. [=*Brassica nigra* (L.) W. Koch.] as the lectotype of the genus *Sinapis*. As a result, nomenclatural synonymy in these two genera, and even across other genera in the subtribe, is common. Taxonomic realignments will be required at both the generic and subtribal levels in order to more accurately reflect genetic relationships. Two possible options exist: (i) expand the genus *Brassica* to include related genera, recognizing the two lineages as subgenera [note: percent divergence across the two lineages as calculated from our cpDNA data is ca. 3%, which is consistent with values for other genera (Palmer 1987]; or (ii) redefine the genus *Sinapis* to include *S. pubescens*, *S. arvensis*, *S. alba*, and three species of *Brassica* (*B. nigra*, *B. fruticulosa*, and *B. tournefortii*). Further studies are in progress to test the monophyletic origins of other genera in the subtribe, a requirement before taxonomic revision of the subtribe can be completed.

In conclusion, a high level of congruence was observed between recognized cytodemes or crossing groups in the Subtribe Brassicinae and the clusters defined by the cpDNA data. A similar congruence has been observed by Doyle et al. (1990) for wild perennial relatives of *Glycine* subgenus *Glycine*. This correlation is significant because of the potential predictive value of cpDNA data in delimiting cytodemes and/or in detecting potentially new breeding material. The observed low levels of variation within the cytodeme group enhance the usefulness of cpDNA data in future systematic studies of intercytodeme relationships in the subtribe.

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Appendix

Chloroplast DNA restriction site and length mutations for each of 20 restriction endonucleases. For each mutation, taxa listed exhibit fragment pattern to right of hyphen; i.e., two fragments separated by symbol + indicate a site gain, whereas a single fragment, a site loss. Fragments bracketed [ ] to left of hyphen are not shown on map. Taxa codes are given in Table 1. Symbols BC(=BC1, 2), BO(=BO1, 2, BA), BRV(=BR, BV, BP, BM), SA(=SA1, 2, SF), SP(=SP1–5), RRS(=RR, RS), unless otherwise indicated. All mutations are defined relative to clones (Cl.) and restriction maps given in Fig. 1; site number and fragment position are ordered from left to right on map. Length mutations (insertion = Ins.; deletion = Del.) are given for only one enzyme and are indicated in Fig. 1 for others

Site	Cl.	Mutation	Taxa
BamHI-			
2	3	7.4 - 6.3 + 1.1	SP3
6	2	2.6 + 4.8 - 7.4	BN,SO,BC,RRS,BO,BB,BG,BRV, BX,BD,ES,CC,DT,DE
7	2	7.4 - 4.4 + 3.0	RRS
9	1	3.5 - 2.0 + 1.5	BT
--	1	4.8, 3.5 - 5.1, 3.2	EC
11	1	7.4 - 1.3 + 6.1	SP
--	6	Del. 0.3: 2.8 - 2.5	SP
20	7	1.1 - 0.7 + 0.4	BG
21	7	1.1 + 0.8 - 1.9	BT
25	10	0.5 + 5.5 - 6.0	BN
26	10	5.5 - 0.5 + 5.0	BX
27	10	5.5 - 2.3 + 3.2	BF
29	10	10.6 - 1.2 + 9.4	SP,OT
30	12	10.6 - 2.1 + 8.5	ES
33	14	4.6 - 3.1 + 1.5	SA
34	14	4.6 + 6.8 - 11.4	BC,BO,BRV,BD
--	14	Ins. 0.4: 4.6 - 5.0	DE
38	18	1.3(1.5)+1.8-3.1(3.3)	BF,DT
39	18	1.8+2.8[5.2]-4.6(7.0)	BN,DE
40	18	2.8[4.6]+2.6-5.4(7.0)	SR,BT,DE
--	18	Del. 0.2: 2.6 - 2.4	SO,BC,RRS,BO,BB,BG,BRV BX,BD,ES,DT,DE
43	19	1.2 + 0.8 - 2.0	CC
46	19	9.2 - 4.6 + 4.6	BF
47	19	9.2 - 5.9 + 3.3	SR,BN
48	19	9.2 - 6.8 + 2.4	BR,CC
49	19	[3.3] - 2.8 + 0.5	SR
50	19	9.2 + 2.7 - 11.9	BC
51	19	9.1 - 2.7 + 6.4	BC,BO,BV,BP,BM
52	20	9.1 - 4.0 + 5.1	BB
54	20	3.4 - 2.5 + 0.9	BF
57	22	4.0 - 0.8 + 3.2	SP
BclI-			
2	3	1.7 + [5.5] - 7.2	SP,HI
3	3	[5.5] - 1.7 + 3.8	SO,RRS,BT,BB,BG,BV,BP,BM, BD,RE,EC,DE
5	1	2.6 + 3.7 - 6.3	BC,BO,RE
10	6	1.9 - 1.4 + 0.5	BRV
18	8	2.6 + 1.4 - 4.0	RE
--	8	Ins. 0.1: 1.4 - 1.5	SO,BC,RRS,BO,BB,BG,BRV BX,BD,RE,DE
22	12	2.3+4.8(9.7)-7.1(12.0)	SO,BC,RRS,BO,BF,BT,BB,BRV BD,ES,EC,DT,DE
23	12	9.7 - 4.8 + 4.9	SA,BN,HI,EC,CC

Site	Cl.	Mutation	Taxa
BclI-			
25	13	4.3 - 3.7 + 0.6	RRS
--	13	4.3 - 4.6	ES
--	14	Ins. 0.3: 2.7 - 3.0	SR,CC
28	14	4.0 - 2.9 + 1.1	ES
30	18	[16.3] - 4.3 + 12.0	EC,OT
31	18	[16.3] - 14.0 + 2.3	SA,SR,BN
33	18	0.5 + 1.8 - 2.3	SO,BC,RRS,BO,BF,BB,BRV,BX,BD,DE
34	18	[2.3] + 2.5 - 4.8	BB,RE
37	19	2.0 + 10.0 - 12.0	SO,BC,RRS,BO,BF,BB,BRV,BX,BD,DE
38	19	10.0 - 4.0 + 6.0	BN
39	19	10.0 - 6.5 + 3.5	RE
40	19	10.0 - 7.6 + 2.4	HI
42	19	5.5 - 1.0 + 4.5	DT
43	20	5.5 + [5.1] - 10.6	BP,DE
44	20	[5.1] - 3.6 + 1.5	SP,BT
--	20	1.6 - 1.7	BC,BO,BRV
47	21	1.6 - 0.9 + 0.7	SA
48	21	1.6 + 1.4 - 3.0	DE
BglI-			
4	10	[27.2] - 20.0 + 7.2	SO,BC,RRS,BO,BB,BG,BRV,BX,BD,ES,DT
8	20	[21.3] - 6.3 + 15.0	BC,BO
BglII-			
1	3	1.0 + 4.8 - 5.8	BO
2	3	4.8 - 0.8 + 4.0	SA
3	2	4.8 + 2.0 - 6.8	CC
4	2	2.0 - 1.2 + 0.8	SP,BT,HI,EC
5	2	2.0 + 1.5 - 3.5	RRS
9	4	4.4 - 3.5 + 0.9	BB
17	7	3.6 - 3.0 + 0.6	BG
24	8	6.8 - 3.0 + 3.8	BB,DT
26	10	2.3 + 0.8 - 3.1	BG
27	12	0.8 + 5.1 - 5.9	SP1,3-5,BT,BD,CC
28	12	5.1 - 3.3 + 1.8	EC
29	12	5.1 + 3.0 - 8.1	SO,RRS
30	12	3.0 - 2.8	BB,BG,ES
31	12	3.0 + 0.6 - 3.6	CC
34	13	2.1 - 1.8 + 0.3	BO
36	14	7.0 - 5.0 + 2.0	EC
38	14	2.0 - 1.3 + 0.7	BN
39	14	9.0 - 2.0 + 7.0	SP,BN
43	18	2.2 + [3.0] - 5.2	BT,EC
44	18	2.0 + 1.0 - 3.0	SP,SO,BT,EC
--	19	Del. 0.3: 4.3 - 4.0	SO,BC,RRS,BO,BB,BG, BRV,BX,BD,ES,DT,DE
46	19	4.0 + 2.4 - 6.4	ES
--	19	Del 0.3: 2.3 - 2.0	SP,BB
52	19	0.8 + 0.7 - 1.5	BT
54	19	0.5 + [2.1] - 2.6	BC,BO
55	19	1.0 + 2.7 - 3.7	SO,BC,RRS,BO,BB,BRV,BX, BD,RE,ES,CC,DT,OT,DE
56	19	[3.7] - 2.1 + 1.6	BC,BO
59	20	9.4 - 3.3 + 6.1	HI
BstEII			
2	3	7.7 - 5.0 + 2.7	BB
4	2	3.6 + 9.4 - 13.0	BD,OT
10	12	7.5 - 4.3 + 3.2	BX
11	12	7.5 + 9.2 - 16.7	ES
--	14	5.3 - 5.0	SR,BN,BC,RRS,BO,BRV,HI,OT
14	17	18.0 - 4.5 + 13.5	CC
ClaI-			
3	2	0.7 + 6.0 - 6.7	BX
4	2	6.0 - 1.0 + 5.0	BF,EC
5	2	6.0 - 1.5 + 4.5	BG
7	1	5.5 - 3.4 + 2.1	SO,BC,RRS,BO,BF,BB,BX
8	1	2.1 + 0.7 - 2.8	BC,BO,BF
9	1	0.7 + 0.8 - 1.5	BB,RE
--	6&7	1.5, 1.8 - 1.2, 2.1	SP
14	7	5.5 + 0.3 - 5.8	SR,BN
16	7	2.0 - 1.1 + 0.9	SO,BC,BO,RRS,BB,BG,BRV
21	8	1.5 + 0.8 - 2.3	SP
23	10	2.6 + 7.2 - 9.8	BC
24	10	10.7 - 4.7 + 6.0	ES
25	10	10.7 - 7.2 + 3.5	SO,BC,RRS,BO,BRV,BD,EC,DT,DE
27	12	2.1 - 1.8 + 0.3	SO,BC,RRS,BO,BRV,BD,EC,DT,DE
28	12	2.1 + 0.9 - 3.0	SP,BT,HI
29	12	0.9 - 0.7 + 0.2	CC
31	12	2.0 - 1.1 + 0.9	SO
34	13	[3.7] - 0.7 + 3.0	SO,BB
36	14	3.3 - 1.0 + 2.3	SP

Site	Cl.	Mutation	Taxa
ClaI-			
37	14	3.3 + 2.5 - 5.8	BC,BO,BRV,OT,DE
38	14	2.5 - 1.6 + 0.9	BT,DT
39	14	2.5 - 1.7 + 0.8	BG,ES
40	14	2.5 - 2.4	HI
42	14	1.2 - 0.8 + 0.4	EC
--	14	1.2, 0.7 - 1.1, 0.8	BB
43	14	1.2 + 0.7 - 1.9	SR,HI
45	17	9.1 - 3.6 + 5.5	ES,DT
47	18	4.7 - 2.8 + 1.9	SA
48	18	4.7 - 4.2 + 0.5	RRS
49	18	7.0 - 4.7 + 2.3	SO,BC,RRS,BO,BB,BRV,BX,BD,ES,DT,DE
50	18	[10.0] - 5.7 + 4.3	BF
51	18	2.3 + 2.8 - 5.1	SO,BC1,OT
52	18	[5.1] + 0.8 - 5.9	SO,BG,BRV,RE,EC
55	19	1.7 + 1.7 - 3.4	DE
56	19	1.7 + 2.2 - 3.9	SR,BN,BF,BT,BX,HI,OT
57	19	2.2[3.9]+6.0-8.2(9.9)	SR,BT,DT
58	19	6.0 - 3.6 + 2.4	DE
60	19	2.0 + 9.4 - 11.4	SP,SR,BN,BT,BRV,BD,RE, ES,EC,CC,DT,DE
62	20	4.8 - 2.1 + 2.7	SO,BD
63	20	4.8 + 2.6 - 7.4	SP1,RRS,BT,BG,ES,OT
64	20	2.6 - 2.3 + 0.3	SR,BN,BB,RE
65	21	2.6 - 2.0 + 0.6	HI
67	22	1.6 + 0.5 - 2.1	SO,BC,RRS,BO,BB,BG,BRV,BX, BD,RE,ES,CC,DT,OT,DE
DraI-			
2	3	1.3 - 1.0 + 0.3	BB,RE
3	3	1.3 + 0.8 - 2.1	BC,BO,DT,DE
5	1	1.5+0.6[1.3]-2.1(2.8)	CC,OT
9	2	2.1 + 1.3 - 3.4	BG
10	2	1.3 - 0.7 + 0.6	SO,BC,RRS,BO,BRV,BX,BD,RE,ES,DE
15	1	1.8 + 1.7 - 3.5	SP,SR,BN,HI
19	4	12.5 - 2.1 + 10.4	EC
20	5	12.5 - 6.5 + 6.0	SR,BN
22	7	9.4 - 5.6 + 3.8	BF
23	7	9.4 - 8.7 + 0.7	BB
24	7	9.4 - 3.6,1.1,3.0,2.3	ES
25	7	9.4 - 6.4 + 3.0	DE
26	7	11.6 - 9.4 + 2.2	SO,BC,RRS,BO,BB,BG,BRV, BX,BD,ES,DT,DE
27	7	2.2 - 1.8 + 0.4	BF,BG
29	8	4.8 - 2.0 + 2.8	RRS,BD,RE,ES
34	10	3.2 - 2.0 + 1.2	BO,OT
35	10	3.2 - 1.5 + 1.7	DE
36	10	3.2 - 1.9 + 1.3	BG
37	10	3.2 - 2.1 + 1.1	BC,BRV
38	10	3.2 - 2.6 + 0.6	SP,HI
40	12	2.8 - 1.8 + 1.0	BT
41	12	2.8 - 2.3 + 0.5	CC
43	12	1.7 - 1.1 + 0.6	SR,DE
45	12	1.2 + 2.0 - 3.2	SO,BC,RRS,BO,BB,BD,DT,DE
--	12	[3.2] - 2.8	BC,BO
46	12	2.0 - 1.3 + 0.7	SR
47	12	2.0 + 0.8 - 2.8	SP
49	12	5.2 - 1.2 + 4.0	RE
50	14	5.2 + 5.2 - 10.4	SP,BT,HI
51	14	5.2 - 2.0 + 3.2	SR,HI
52	14	5.2 - 2.3 + 2.9	BC,BO,BRV,DT
53	14	[2.9] - 2.4 + 0.5	BC
55	14	1.2 - 0.7 + 0.5	BN
59	17	8.5 - 2.8 + 5.7	SA
60	17	8.5 - 3.5 + 5.0	DT
62	18	[3.5] - 3.0 + 0.5	SA,SR,BN,BF,BG
64	18	1.6 - 0.9 + 0.7	SR
66	18	1.7 - 1.0 + 0.7	BO,BT,BRV,BX,BD,ES,CC
67	18	1.7 - 1.2 + 0.5	SR,BG
68	18	1.7 - 1.1 + 0.6	RR
69	18	1.7 - 1.4 + 0.3	SP
71	18	1.4 + 1.8 - 3.2	ES,DT
72	19	1.8 + 1.1 - 2.9	SP,BC,BO,BT,BRV,ES,HI,DE
76	19	2.4 - 1.3 + 1.1	SO,RRS,BF,BT
77	19	2.4 - 1.8 + 0.6	BX,CC
--	19	2.4, 3.8 - 3.0, 3.2	DT
79	19	3.5 - 2.0 + 1.5	BX
80	19	3.5 + 0.8 - 4.3	BF
81	19	0.8 + 0.9 - 1.7	BN
86	20	3.0 - 2.0 + 1.0	SO,BC,BO,BG,BRV,BX,BD, ES,CC,DT,OT,DE
87	20	3.0 + 0.6 - 3.6	BN
89	20	1.2 - 1.0	DE
92	20	[1.6 + 0.7] - 2.3	SO,BC,BO,RRS,BB,BG,BRV, BX,BD,ES,DT,DE

Site	Cl.	Mutation	Taxa
DraI-			
93	20	2.3 - 1.8 + 0.5	BC,BO
95	21	1.5 + 1.8 - 3.3	BB,OT
96	21	[3.3] - 2.5 + 0.8	BB,OT
EcoRI-			
4	3	[2.8] - 2.0 + 0.8	BC,BG,BRV,EC
5	3	0.8[2.8]+3.7-4.5(6.5)	BC,BO,BRV,HI,EC,DT,DE
10	1	2.4 - 1.9 + 0.5	BA
11	1	2.4 + 0.8 - 3.2	SA,BC
12	1	0.8 + 1.2 - 2.0	BD
13	1	1.2 + 1.1 - 2.3	RRS
21	6	1.4 + 0.5 - 1.9	BC,BO,BX
26	7	1.6 + 1.5 - 3.1	BG
28	7	[1.5] - 0.8 + 0.7	SO,BC,RRS,BO,BRV,BX,BD,ES,DT,DE
33	10	1.4 + 0.7 - 2.1	SO,BC,RRS,BO,BB,BRV,BX,BD, RE,ES,EC,DT,DE
34	10	0.7 + 0.6 - 1.3	SP
35	10	0.6 + 2.4 - 3.0	BB
36	10	2.4 - 1.0 + 1.4	SP,SA,BN
37	10	2.4 - 1.3 + 1.1	BX
38	10	2.4[1.4]+2.7-5.1(4.1)	SP,BT,BD,HI,EC
39	12	2.7 + 2.3 - 5.0	SO,BC,RRS,BO,BB,BG,BRV,BX, BD,ES,HI,EC,DT,OT,DE
--	12	2.0 - 1.8	SO,BC,BO,BG,BRV,ES,DT,DE
44	12	2.0 + 1.9 - 3.9	BB
49	14	0.9 + 0.8 - 1.7	SP,BO,BF,BG,BD
52	14	7.4 - 0.6 + 6.8	SA
53	14	7.4 - 1.4 + 6.0	BB
54	14	7.4 - 3.1 + 4.3	BT,OT
55	17	7.4 + 7.5 - 14.9	EC
58	18	1.4 - 1.0 + 0.4	BC,BO,RRS,BRV,BX,BD,ES
59	18	1.4 - 0.9 + 0.5	SR,BN,BF,BT,EC
60	18	1.4 - 1.2	SA
61	18	1.4 - 0.8	SO
--	18	2.4 - 2.1	SP,BC,RRS,BO,BRV,BX,ES,EC,DE
--	19	Del. 0.2: 4.0 - 3.8	SR,BT,HI
67	19	1.2 + 0.9 - 2.1	SR
68	19	0.9 + 1.6 - 2.5	ES
69	19	1.6+2.8[1.3]-4.4(2.9)	BF,BB,BX,EC
70	19	2.8 - 1.3 + 1.5	SO,BC,BO,RRS,BG,BRV,BD,ES,DT,DE
71	19	2.8+2.7(5.0)-5.5(7.8)	BN,BB,BX,DE
72	19	5.0 - 0.7 + 4.3	BC,BO,BRV,DE
73	19	2.7 - 1.9 + 0.8	CC
74	19	5.0 - 2.7 + 2.3	BN,BT,CC
78	20	1.9 + 1.8 - 3.7	HI,DT
80	20	2.4 + 0.5 - 2.9	SA,SO,BD,ES
-	20	[2.9] - 2.7	SO
-	21	1.6 - 1.5	DT
85	22	[1.9] - 1.0 + 0.9	SO,BC,RRS,BO,BRV,BX,BD, RE,ES,EC,DT,DE
EcoRV-			
1	3	14.0 - 13.0 + 1.0	BB
2	3	14.0 + 13.0 - 27.0	RRS
3	3	13.0 - 3.0 + 10.0	BC,BO,BA
4	1	13.0 - 12.2 + 0.8	DT
5	1	13.0 + 14.0 - 27.0	HI
6	1	14.0 - 3.0 + 11.0	RR
7	6	14.0 + 1.1 - 15.1	BX
15	8	4.4 + 11.6 - 16.0	BT,BB,BX
16	10	11.6 - 2.3 + 9.3	ES
--	12	3.0 - 2.8	CC
21	14	2.3 - 2.0 + 0.3	SA,SR,BN,BF
23	14	3.8 - 3.2 + 0.6	BC,BO,BRV
25	14	1.1 - 0.6 + 0.5	SR,HI
26	14	1.1 + 1.6 - 2.7	BB
--	14	1.6 - 1.5	BC,BO,BRV
29	18	8.6 - 6.4 + 2.2	BG
30	18	8.6 - 7.1 + 1.5	BF
31	18	8.6 + 2.0 - 10.6	BT
32	18	2.0+8.2(8.7)-10.2(.7)	SA,BG,DT,DE
33	16	8.2 - 5.4 + 2.8	SP,BRV
34	19	[2.8] - 1.4 + 1.4	BRV
35	19	2.3(10.2)+.5-2.8(10.7)	SP,DE
37	19	3.2 - 1.7 + 1.5	BX
38	19	3.2 + 0.8 - 4.0	RR
40	20	11.0 - 7.0 + 4.0	BB,DT
41	20	11.0 + 1.3 - 12.3	SA,RS,BD,OT
--	20	1.3 - 1.1	SO,BC,RRS,BO,BB,BRV,BX,BD,ES,DE
43	20	9.0 + 4.5 - 13.5	BT,BB,BX,ES

Site	Cl.	Mutation	Taxa
HindIII-			
2	2	1.4 - 1.0 + 0.4	RE
3	2	1.4 + 9.0 - 10.4	RRS,DT,OT
4	2	9.0 - 6.0 + 3.0	BD
6	1	4.6 - 4.0 + 0.6	BN
7	1	4.6 + 1.5 - 6.1	SO,BC,RRS,BO,BG,BRV,BX, BD,RE,ES,HI,DT,OT,DE
8	1	6.1 - 5.5 + 0.6	RE
9	1	1.5(6.1)+2.7-4.2(8.8)	BF,BD,OT
17	8	0.6 + 10.0 - 10.6	SA
18	8	10.0 - 0.9 + 9.1	HI
19	10	10.0 - 7.7 + 2.3	BR
25	12	6.8 - 4.8 + 2.0	SO,BC,RRS,BO,BB,BG, BRV,BX,BD,ES,DT,DE
27	14	13.0 - 6.3 + 6.7	BF
28	11	13.0 + 9.0 - 22.0	SA,SR,BN
29	18	9.0 - 6.6 + 2.4	BF
--	18	2.6 - 2.4	SO
--	18	2.8, 2.6 - 2.7, 2.7	BC,OT
32	18	2.6 + 0.6 - 3.2	RE
35	19	7.0 + 3.3 - 10.3	BT
36	19	3.3 - 2.1 + 1.2	DT
37	19	3.3 + 1.6 - 4.9	BC,BO,RE
39	20	1.6 - 1.3 + 0.3	BC,BO,BRV
41	20	3.0 - 2.3 + 0.7	RE
-	20	3.0 - 2.9	BP
42	20	3.0 + 3.5 - 6.5	DE
43	20	3.5 + 7.0 - 10.5	CC
KpnI-			
5	12	4.3 + 7.8 - 12.1	SO,BX,BD
6	12	7.8 - 7.3 + 0.5	SP
8	14	16.0 - 2.2 + 13.8	CC
NciI-			
1	3	0.45 + 7.0 - 7.45	SA,EC
3	3	0.5 + 2.5 - 3.0	BN,SO,BB,CC,OT,DE
5	2	0.9 + 1.4 - 2.3	SP,OT
--	2	0.9 - 0.7	BO
--	2	0.9 - 1.2	RRS
6	2	1.4 + 0.8 - 2.2	BR,CC
--	6	2.4 - 2.6	BC,BO
9	1	0.45 + 0.5 - 0.95	BG,EC
28	8	1.7 + 1.0 - 2.7	BT
31	10	4.3 - 4.0 + 0.3	SP
32	10	4.3 + 2.1 - 6.4	DT
33	12	2.1 + 2.1 - 4.2	HI
37	12	[2.3] - 0.5 + 1.8	RRS,BX,RE
38	12	1.8 - 0.6 + 1.2	RE
40	12	5.4 - 0.5 + 4.9	BG
41	12	2.7 + 2.7 - 5.4	SO,BC,RRS,BO,BB,BG,BRV, BX,BD,RE,ES,DT,DE
45	14	1.8 - 1.1 + 0.7	CC
46	14	1.8 - 1.4 + 0.4	BF
47	14	[2.3] - 1.8 + 0.5	SA,SR,BN,BF,RE,CC
48	14	[2.3] + 3.7 - 6.0	BT
50	11	2.7 - 0.5 + 2.2	BG,DT
51	17	2.7 - 1.4 + 1.3	SP
52	17	2.7 + 1.3 - 4.0	BF,BX,EC
56	18	1.7 - 0.85 + 0.85	DT
58	18	3.6 - 2.8 + 0.8	BG,DE
63	19	2.4 + 1.1 - 3.5	RE
67	19	1.6 + 2.3 - 3.9	BO
68	19	14.9 - 2.3 + [12.6]	SO,BC,RRS,BO,BB,BG,BRV, BX,BD,ES,DT,DE
69	19	14.9 - 4.0 + 10.9	SR,BT
70	19	[12.6] - 3.2 + 9.4	RRS,BT,BB,BG,BRV,BX,ES,DT,DE
71	19	14.9 - 8.5 + 6.4	SP
72	19	9.4 - 5.4 + 4.0	DT
--	20	Ins. 0.4: 5.0 - 5.4	DT
75	20	1.6 + 0.4 - 2.0	RE,EC
77	21	4.0 + 1.0 - 5.0	BT
PstI-			
2	2	4.9 - 3.2 + 1.7	RE
3	1&2	4.9 + 18.7 - 23.6	BO
7	12	13.0 + 2.6 - 15.6	SO,BC,RRS,BO,BB,BG,BRV, BX,BD,RE,ES,DT,DE
8	12	2.6 + 16.6 - 19.2	BB,BG,CC
9	12	16.6 - 2.0 + 14.6	BX
10	14	16.6 + 1.25 - 17.8	ES
14	21	[30.4] - 24.4 + 6.0	ES
15	21	[30.4] - 28.4 + 2.0	SO,BC,RRS,BO,BB,BG,BRV,BX,BD,DT,DE

Site	Cl.	Mutation	Taxa
SacI-			
13	16	1.05 + 1.10 - 2.15	RS
15	19	15.0 - 5.0 + 10.0	DT
SalI-			
3	10	4.1 + 5.2 - 9.3	SO,BC,RRS,BO,BT,BB,BRV,BX, BD,RE,ES,CC,DT,OT,DE
5	12	10.0 + 47.0 - 57.0	RRS
7	20	16.0 - 12.2 + 3.8	CC
ScaI-			
1	3	14.0 + 5.7 - 19.7	BX
2	2	5.7 - 3.2 + 2.5	DT
4	2	3.5 + 20.0 - 23.5	RE
7	8	2.8 + 0.4 - 3.2	RRS
11	10	13.0 - 2.0 + 11.0	BG,BD
12	12	11.0 - 5.8 + 5.2	BG
15	14	5.0 - 1.1 + 3.9	RRS,BB,BX
16	14	5.0 - 4.2 + 0.8	SO
17	14	5.0 + 1.0 - 6.0	SP,HI,EC,CC
18	14	1.0 + 4.6 - 5.6	RRS,BT,BG,DT
21	16	1.8 + 2.2 - 4.0	BT
22	19	2.2 + 9.0 - 11.2	SR,BN
23	19	9.0 - 3.8 + 5.2	BG
25	19	1.2 + 3.0 - 4.2	DT
26	19	3.0 + [19.5] - 22.5	BX
27	20	[17.0] + 5.5 - 22.5	BX
SmaI-			
2	1	[26.0] - 16.0 + 10.0	SR,BN
4	6	3.1 + 0.9 - 4.0	BC,BO
7	8	1.5 + 1.0 - 2.5	BT
9	10	[50.0] - 9.0 + 41.0	SO,BC,RRS,BO,BB,BRV, BX,BD,ES,DT,DE
StuI-			
1	3	5.0 + 18.0 - 23.0	EC,CC,OT
2	1	18.0 - 11.0 + 7.0	RRS
9	10	15.0 + 9.0 - 24.0	BX
10	12	9.0 + 6.5 - 15.5	BT
11	13	6.5 + 15.0 - 21.5	SO,BC,RRS,BO,BB,BG,BRV, BX,BD,ES,DT,DE
14	18	2.1 + 6.6 - 8.7	SR,BN
15	18	6.6 - 3.0 + 3.6	DE
--	18	2.1, 6.6 - 1.9, 6.8	RE
19	20	8.5 + 4.3 - 12.8	BB,BG
20	20	4.3 + 7.0 - 11.3	BD,RE
22	21	1.4 + 9.0 - 10.4	BC,BO,RE,OT

Site	Cl.	Mutation	Taxa
XbaI-			
1	3	[13.9] + 6.0 - 19.9	DE
2	2	6.0 - 3.2 + 2.8	EC
3	2	6.0 - 4.8 + 1.2	ES
5	2	1.7 - 0.9 + 0.8	BF
6	2	1.7 - 1.3 + 0.4	SP,SO,BC,RRS,BO,BT,BB,BG, BRV,BX,HI,ES,CC,RE,DT,DE
--	1	2.1 - 2.0	CC
9	1	9.4 + 4.5 - 13.9	SO,BC,BO
10	1	[13.9] + 4.5 - 18.4	BC,BO
12	7	5.3 - 3.5 + 1.8	HI
17	10	2.6 + 4.1 - 6.7	DT
18	10	4.1 + 1.5 - 5.6	BF,RE
19	10	1.5 + 6.7 - 8.2	BX
20	12	6.7 + 1.0 - 7.7	BF,CC
22	13	6.5 - 5.1 + 1.4	BF
23	12	6.5 + 0.9 - 7.4	BG,DT
24	14	0.9 - 0.5 + 0.4	BX
25	14	0.9 + 1.6 - 2.5	SP
26	14	1.6 + 2.4 - 4.0	BG
28	14	16.4 - 2.8 + 13.6	DE
29	11	16.4 - 6.4 + 10.0	BB
30	17	16.4-12.0+(3.7+0.7)	BC
31	17	16.4-(12.0+3.7)+0.7	BC
33	18	4.3 - 2.6 + 1.7	SA,SR,BN
34	18	4.3 - 2.8 + 1.5	EC
35	18	(4.1) + 1.0 - 5.1	BG,RE,DE
39	19	4.0 - 2.5 + 1.5	RE
41	19	7.0 - 6.2 + 0.8	SO
42	19	7.0 + [4.7] - 11.7	BD
43	20	[4.7] - 2.2 + 2.5	BC,BO,DE
45	20	1.8 - 1.1 + 0.7	SO,BC,RRS,BO,BRV,BX,DT,OT,DT,DE
47	20	[3.4] - 1.8 + 1.6	SR,BN
--	20	1.8,1.6 - 1.9,1.5	SO,BC,RRS,BO,BB,BRV,BX,ES,DT,DE
49	20	1.7 - 0.9 + 0.8	SO,BC,RRS,BO,BB,BRV,BX,ES,DT,DE
50	20	1.7 + [3.5] - 5.2	HI
51	20	[3.4] + [1.8] - 5.2	HI
52	20	1.2+0.6[4.3]-1.8(5.5)	SR,BN,HI,OT
53	20	0.6 + 3.7 - 4.3	SA,SR,BN,BF
XhoI-			
2	1	17.1 - 11.1 + 6.0	BRV
3	1	15.3 + 9.8 - 25.1	SP,BT,RE,EC,CC,OT
--	1-3	15.3,9.8 - 17.1,8.0	SO,BC,RRS,BO,BB,BRV,BX,BD,ES,DT,DE
8	10	4.1 + 5.8 - 9.9	DT
11	14	9.8 - 5.8 + 4.0	BG,DT
12	14	9.8 - 6.6 + 3.2	SP
13	14	9.8 - 9.0 + 0.8	BB
14	14	[4.0] + 11.1 - 15.1	DT
15	17	11.1 - 7.0 + 4.1	BX
17	19	20.0 + 15.0 - 35.0	EC
20	20	2.2 + 6.5 - 8.7	SO,BC,RRS,BO,BB,BRV,BX,BD,CC,DE
21	21	6.5 - 2.8 + 3.7	EC,OT

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