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Molecular systematics of *Brassica* and allied genera (Subtribe Brassicinae, Brassiceae) – chloroplast DNA variation in the genus *Diplotaxis*

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Summary. Chloroplast DNA restriction site variation for 17 endonucleases was surveyed for the large single-copy region of the genome in 26 taxa of the genus *Diplotaxis* and compared with previously mapped site mutations in other members of the Subtribe Brassicinae (Tribe Brassiceae, Cruciferae). A total of 259 restriction site and length mutations were observed, 206 (80%) of which showed variation at the interspecific level. Phylogenetic analysis indicated a clear division of the genus *Diplotaxis* into the same two lineages for the Subtribe Brassicinae, described previously by Warwick and Black as Rapa/Oleracea and Nigra. Levels of genetic divergence and taxon groupings suggested by the restriction site variation were highly consistent with previously recognized cytodemes or crossing groups and the geographical distribution of *Diplotaxis* taxa. However, the data were inconsistent with the morphologically based taxonomic delimitation of the genus, certain subgeneric circumscriptions, and even species delimitation in the case of *D. virgata*. *Diplotaxis* species were separated into three major groups in each of the two lineages. In the Rapa/Oleracea lineage, Group A included *D. erucoides* ssp. *erucoides* and *D. cossoniana*. Group B included two subgroups: B (i) *D. tenuifolia*, *D. cretacea*, and *D. simplex* and B (ii) *D. harra*. Group C included *D. viminea* and *D. muralis*. Within the Nigra lineage, group D included *D. siettiana*, *D. ibicensis*, *D. brevisiliqua*, and *D. gomez-campoi*. Group E included *D. brachycarpa*. Group F included three subgroups: F (i) *D. assurgens*, *D. tenuisiliqua*, an accession of *D. virgata* from southern Morocco (DVA), and *D. siifolia*; F (ii) *D. berthautii* and *D. virgata* f. *sahariensis*; and F (iii) *D. catholica*, *D. catholica* var. *rivulorum*, *D. virgata* ssp. *virgata*. These groups often showed greater genetic closeness to other species from other genera in the Subtribe than to other species of *Diplotaxis*.

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

Introduction

Both chloroplast DNA (cpDNA) (e.g., Erickson et al. 1983; Palmer et al. 1983; Yanagino et al. 1987; Warwick and Black 1991) and nuclear DNA (Song et al. 1988a, b, 1990; Hosaka et al. 1990) studies have contributed to a clarification of genetic relationships among *Brassica* and allied genera. Based on cpDNA restriction site variation, Warwick and Black (1991) provided evidence for two distinct lineages in Subtribe Brassicinae (including the genera *Brassica*, *Coincya*, *Diplotaxis*, *Eruca*, *Erucastrum*, *Hirschfeldia*, *Raphanus*, and *Sinapis*). The two lineages, designated Rapa/Oleracea and Nigra, appear to have diverged early in the evolution of the subtribe, and prior to the evolution of distinct cytodemes. In addition, levels of genetic divergence in the chloroplast genome were found to be highly consistent with previously recognized cytodeme or crossing groups in the subtribe (Harberd 1976; Takahata and Hinata 1983), but provided evidence for the polyphyletic nature of both *Brassica* and *Sinapis* (Warwick and Black 1991).

The objective of the present study was to test proposed species and cytodeme relationships within *Diplotaxis* and to examine their evolutionary relationship to other taxa in Subtribe Brassicinae using cpDNA restriction site variation. The generic delimitation of the genus *Diplotaxis* and its relationship with its nearest relatives in the subtribe Brassicinae have not been established adequately (Al-Shehbaz 1985). Genera are defined on the basis of few morphological traits; for example, *Diplo-*

taxis is separated from *Brassica* primarily on the basis of biseriate, small, generally ovoid or ellipsoidal seeds; in *Brassica* seeds are usually uniseriate, larger, and globose (Schulz 1919; Tutin et al. 1964; Maire 1965; Al-Shebaz 1985). However, boundaries between the two genera, become less sharply defined as a few *Brassica* species have biseriate or at least subbiseriate seeds (Tutin et al. 1964; Al-Shebaz 1985). Cytogenetically, *Diplotaxis* is a component of the *Brassica* "coenospecies," i.e., taxa capable of artificial hybridization with the six cultivated species of *Brassica* as well as other genera in the subtribe Brassicinae: *Coincya*, *Erucastrum*, *Hirschfeldia*, *Sinapidendron*, and *Sinapis* (Harberd 1976; Harberd and McArthur 1980; Takahata and Hinata 1983). Given the morphological and hybridization data, and the results from earlier molecular systematic studies of *Brassica* and *Sinapis*, it was therefore important to study the interspecific relationships of species of *Diplotaxis*, not only to members of the same genus, but to other closely related genera.

Diplotaxis contains more than 20 species and is mainly distributed in central Europe and the Mediterranean region, particularly in northwest Africa (Schulz 1919; Maire 1965; Greuter et al. 1986; Martínez-Laborde 1988). It has been postulated as basal to the rest of the Brassiceae, as nearly all of the presumed primitive morphological characters for the tribe are present (Gómez-Campo 1980). The morphological characters used to define infrageneric groups in *Diplotaxis* are often highly variable (Al-Shehbaz 1985), and taxonomic treatments of the genus vary, ranging from two up to five subgeneric and sectional divisions (Schulz 1919; Maire 1965; Martínez-Laborde 1988). One tetraploid and 12 diploid cytodemes have been described in the genus (Harberd 1976; Harberd and McArthur 1980; Takahata and Hinata 1983). There is a continuous series of haploid numbers, including n = 7, 8, 9, 10, 11, and 13, with one alloploid species with n = 21 (Table 1, Gómez-Campo and Hinata 1980).

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. Three to six plants were grown in a greenhouse from seeds of each accession. The identity of voucher specimens of mature plants of each accession was verified by the senior author and deposited in the Vascular Plant Herbarium, Biosystematics Research Center, Agriculture Canada, Ottawa. DNAs were obtained from single plants in each accession. All sections of *Diplotaxis* were represented with the exception of Sect. *Hesperidium* O. E. Schulz [includes *D. acris* (Forsk.) Boiss. and *D. griffithii* (Hook f. & Thoms.) Boiss.], for which seed was not available.

Molecular methods

Procedural methods for total cellular DNA extraction, purification, restriction enzyme digestion, filter hybridization, radioactive probing, and autoradiography followed Warwick and Black (1991), except that DNA was digested with each of 17 restriction endonucleases (Appendix). Filters were sequentially probed with 17 clones from the chloroplast genome of *Brassica juncea*. These represent the large single-copy region of the chloroplast genome or approximately 75% of the genome. Restriction site variation for each of the endonucleases was compared with restriction site maps and site and length mutations given in Fig. 1 and Appendix, respectively, in Warwick and Black (1991). All new sites observed were designated with a number corresponding to the previously mapped site immediately to the left and a letter A, B, or C.

In the phylogenetic analysis each mutation was treated as a two-state variable. Phylogenetic analysis of species relationships was conducted using the computer program "Phylogenetic Analysis Using Wagner Parsimony" (PAUP), version 2.4 (Options: Hold = 1, Swap = Alternate, Addseq = Closest, and Mulpars; Rooting = Midpoint), developed by D. L. Swofford (Illinois Natural History Survey). The shortest phylogenetic tree(s) were calculated on the basis of all mutations that were shared by two or more taxa; shared mutations not evident in *Diplotaxis*, but included in the analyses, are as given in Warwick and Black (1991).

Results and discussion

In the *Diplotaxis* taxa surveyed, 259 mutations, including both site and length mutations, were observed (Appendix), with 206 (80%) of these showing variation at the species level. In the present study, very low levels of variation, i.e., 0–0.01%, were found among plants of an accession or a given species (with the exception of *D. virgata*).

The number of mutations unique to a single species is indicated in brackets on the terminal branch points. Ten equally parsimonious trees were obtained (available from the authors on request); these were very similar and showed consistent topologies in terms of both species of *Diplotaxis* included within each of the two lineages and the relative major taxon groupings within each lineage (Groups A to F). A composite tree is represented in Fig. 1. Parallel evolution of several cpDNA mutations (both reversals and convergent parallelisms) appears to have occurred independently in the separate lineages. These affect the consistency index of the tree in Fig. 1 (= 0.45), such that when each lineage was analyzed separately, fewer trees were obtained (three and five for the Nigra and Rapa/Oleracea lineages, respectively) and markedly increased consistency indices were obtained (= 0.60). Again, these trees consistently showed the same major *Diplotaxis* taxon groupings in each lineage. Results are available from authors on request.

Phylogenetic analysis indicated a clear division of the genus *Diplotaxis* into the two evolutionary lineages for the Subtribe Brassicinae, described previously by Warwick and Black (1991) as Rapa/Oleracea and Nigra lineages (Fig. 1, Table 1). Among the 26 taxa of *Diplotaxis* surveyed, three major groups of *Diplotaxis* taxa were

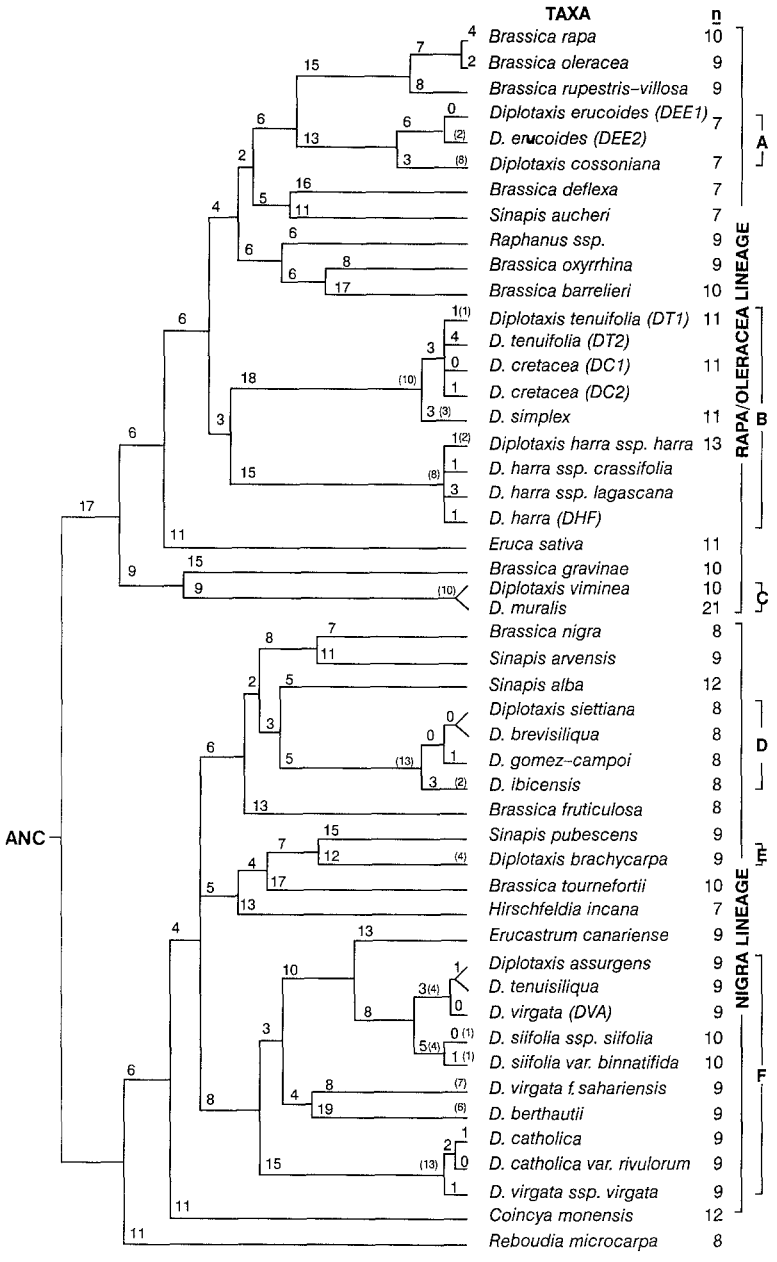

Fig. 1. Selected phylogenetic tree for the genus *Diplotaxis* and related taxa in the Subtribe Brassicinae, based on PAUP analyses of the chloroplast DNA restriction site/length mutations in Appendix, which are shared by two or more taxa/accessions. Tree length is steps, consistency index 0.45. Tree 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 taxon. Mutations unique to a given species are indicated in brackets at end of branch and should be added to determine terminal branch length. ANC shows the common hypothetical ancestor; n = haploid chromosome number

seen in each of the lineages. As was found in earlier cpDNA studies for the subtribe (Warwick and Black 1991), levels of genetic divergence and groupings of taxa were highly consistent with previously recognized cytodemes. However, the data were inconsistent with the morphologically based taxonomic delimitation of the genus, certain subgeneric circumscriptions, and even species delimitation in the case of *D. virgata*.

Rapa/Oleracea lineage

The species of *Diplotaxis* did not form a monophyletic group in the lineage, but were separated into three major groups (Groups A, B, and C) (Table 1, Fig. 1). Group A included *D. erucoides* and *D. cossoniana*. Group B included two subgroups: (i) *D. tenuifolia*, *D. cretacea*, and *D. simplex* and (ii) *D. harra*. Group C included *D. viminea* and *D. muralis*.

The two n = 7 species surveyed, *Diplotaxis erucoides* and *D. cossoniana*, formed a distinct cpDNA group (Group A). The latter taxa has recently been given subspecific rank, i.e. *D. erucoides* ssp. *longisiliqua* (Cosson) Gómez-Campo (Gómez-Campo 1981) and *D. erucoides* ssp. *cossoniana* (Reut.) Mart.-Lab. (Martínez-Laborde 1991 a). Morphologically, the two taxa are separated primarily on the basis of petal color, nervation patterns on petals, and fruit size (Schulz 1919; Maire 1965; Gómez-Campo 1981; Martínez-Laborde 1988). Geographically,

Table 1. Taxa grouped according to two cpDNA lineages and cpDNA groups A to F as in Fig. 1, taxa code, chromosome number (n), and source of seed for plant material included in this study. DNAs were obtained from single plant selections of each accession

Taxa[a]	Code	n[b]	Source[c]
Rapa/Oleracea lineage			
Group A			
D. erucoides (L.) DC. ssp. *erucoides*	DEE		
Accession 1	DEE1	7	BGS No. 330 (BCN 3463)
Accession 2	DEE2	7	GCC 1235-67 (BCN 7009)
D. cossoniana (Reut.) O.E. Schulz	DCS	7	GCC 3659-75 (BCN 7010)
Group B			
Subgroup B (i)			
D. tenuifolia (L.) DC.	DT	11	
Accession 1	DT1	11	BGPa No. 154 (BCN 3416)
Accession 2	DT2	11	GCC 0980-66 (BCN 7000)
D. cretacea Kotov.	DC		
Accession 1	DC1	11	GCC 4189-76 (BCN 7001)
Accession 2	DC2	11	BGV No. 1742 (BCN 3510)
D. simplex (Viv.) Sprengl	DSX	11	GCC 1931-71 (BCN 7002)
Subgroup B (ii)			
D. harra (Forsk.) Boiss.	DH	13	
ssp. *harra*	DHH	13	GCC 1472-68 (BCN 7005)
ssp. *crassifolia* (Raf.) Maire	DHC	13	GCC 5966-81 (BCN 7007)
ssp. *lagascana* (DC.) O. Bòlos & Vigo	DHG	13	GCC 0913-66 (BCN 7008)
Accession: Algeria	DHF	13	GCC 1831-70 (BCN 7006)
Group C			
D. viminea (L.) DC.	DV	10	GCC 2108-76 (BCN 7003)
D. muralis (L.) DC.	DM	21	GCC 0990-68 (BCN 7004)
Nigra lineage			
Group D			
D. siettiana Maire	DST	8	GCC 3025-76 (BCN 7012)
D. brevisiliqua (Coss.) Mart.-Lab.	DBV	8	GCC 7517-87 (BCN 7013)
D. gomez-campoi Mart.-Lab.	DGC	8	GCC 4065-76 (BCN 7014)
D. ibicensis (Font Quer) Gómez-Campo	DIB	8	GCC 3457-76 (BCN 7011)
Group E			
D. brachycarpa Godr.	DBR	9	GCC 6467-84 (BCN 7017)
Group F			
Subgroup F (i)			
D. assurgens (Del.) Gren.	DAS	9	GCC 1120-67 (BCN 7015)
D. tenuisiliqua Del.	DTT	9	GCC 1123-67 (BCN 7025)
D. virgata (Cav.) DC.			
Accession S. Morocco	DVA	9	GCC 3003-74 (BCN 7019)
D. siifolia G. Kunze	DS		
ssp. *siifolia*	DSS	10	GCC 1447-68 (BCN 7023)
var. *bipinnatifida* Cosson	DSB	10	GCC 2970-74 (BCN 7024)
Subgroup F (ii)			
D. virgata (Cav.) DC. f. *sahariensis* Cosson	DVS	9	GCC 5545-80 (BCN 7021)
D. berthautii Br.-Bl. & Maire	DBE	9	GCC 1079-67 (BCN 7016)
Subgroup F (iii)			
D. catholica (L.) DC.			
ssp. *catholica*	DCA	9	GCC 1390-68 (BCN 7018)
var. *rivulorum* (Br.-Bl. & Maire) Maire	DCR	9	GCC 3644-75 (BCN 7020)
D. virgata (Cav.) DC. ssp. *virgata*	DVV	9	GCC 0952-66 (BCN 7022)

[a] The following taxa are listed in the germplasm publication from GCC (Gómez-Campo 1990) under the following names: DCS – *D. erucoides* (L.) DC. spp. *cossoniana* (Reut.) Mart.-Lab.; DHF – *D. harra* (Forsk.) Boiss. ssp. *numidica* Mart.-Lab.; DVA – *D. virgata* (Cav.) DC. ssp. *australis* Mart.-Lab.; and DVS – *D. virgata* (Cav.) DC. ssp. *sahariensis* Mart.-Lab.

[b] Chromosome numbers are from Gómez-Campo and Hinata (1980) and Martínez-Laborde (1988)

[c] BGPa = Botanical Garden, Pavia University, Italy; BGS = Botanical Garden, Salzburg University, Austria; BGV = Botanical Garden, Ecological and Botanical Inst., Vacratot, Hungary; GCC = Gómez-Campo Coll., E.T.S.I.A., Madrid, Spain. Note: Collection number on herbarium labels for specimens deposited at Herbarium, Agriculture Canada, Ottawa, indicated either by GCC collection number or BCN number for collections from other sources

D. cossoniana is restricted to Algeria and Morocco, while *D. erucoides* has a much wider distribution in Europe and the Mediterranean region (Tutin et al. 1964; Gómez-Campo 1981; Greuter et al. 1986; Martínez-Laborde 1988). Although *D. cossoniana* was not studied by Takahata and Hinata (1983), studies by Gómez-Campo (1981) suggested that breeding barriers between the taxa are primarily genetic in nature. In sympatric areas of their range, interfertility was very low and hybrids were completely sterile. Our cpDNA data is consistent with species rank and separate cytodeme status for the two taxa.

The three taxa in the *D. tenuifolia* cpDNA subgroup (n = 11), *D. tenuifolia*, *D. cretacea*, and *D. simplex*, are in the same cytodeme (Harberd 1976; Takahata and Hinata 1983; Martínez-Laborde 1990). *Diplotaxis tenuifolia* and *D. cretacea* are very similar morphologically. The latter taxon differs primarily by having pinnatifid/bipinnatifid leaves and an annual/biennial growth habit (although occasionally it is a short-lived perennial), as compared with *D. tenuifolia*, which is characterized by entire/pinnatifid leaves and a perennial growth habit (Tutin et al. 1964; Martínez-Laborde 1988). Geographically, *D. cretacea* is a narrow endemic in eastern Europe–N.E. Ukraine and adjacent parts of Russia (Tutin et al. 1964), compared to the more widespread and primarily European distribution of *D. tenuifolia* (Schulz 1919; Tutin et al. 1964; Maire 1965; Greuter et al. 1986; Martínez-Laborde 1988). The low levels of cpDNA divergence, the morphological similarity, and the geographical separation of these two taxa are more consistent with subspecific rather than specific rank. *Diplotaxis simplex* is also an annual, but can be distinguished from the latter two taxa by having a decumbent rather than an erect growth habit, and suberect to patent siliques as compared with suberect to erect siliques (Schulz 1919; Maire 1965; Martínez-Laborde 1988). In addition, *D. simplex* is separated geographically, occurring in Algeria, Tunisia, Libya, and Egypt (Maire 1965; Greuter et al. 1986; Martínez-Laborde 1988). Morphologically, *D. simplex* is most similar to the alloploid *D. muralis*, and this is reflected by the placement of the latter two taxa and *D. viminea* in Section *Anocarpum* by Schulz (1919).

The sister group to the *D. tenuifolia*/*D. simplex* subgroup (n = 11) was the *D. harra* subgroup (n = 13). *Diplotaxis harra* and *D. crassifolia* (Raf.) DC. belong to the same cytodeme in the subtribe (Harberd 1976; Takahata and Hinata 1983). Little divergence of the chloroplast genome was detected among the four accessions of *D. harra*, consistent with the subspecific taxonomic ranking assigned to these taxa (Maire 1965; Bòlos and Vigo 1974; Martínez-Laborde 1991 b). Geographically, *D. harra* ssp. *harra* is widely distributed across northern Africa and the Middle East, while the other three subspecies have restricted distributions, i.e., ssp. *crassifolia* – Sicily, ssp. *lagascana* – Spain, and accession DHF – Algeria (Tutin et al. 1964; Greuter et al. 1986; Martínez-Laborde 1988).

No mutational differences were observed within the group containing *D. viminea* and *D. muralis*. *Diplotaxis viminea* (n = 10) is considered a separate cytodeme (Harberd 1976; Takahata and Hinata 1983). Previously recognized as one parent of the alloploid *D. muralis* (n = 10 + 11) (Harberd and McArthur 1972), the cpDNA data confirmed *D. viminea* as the maternal parent. Their geographical distribution in Europe and the Mediterranean region is similar (Tutin et al. 1964; Greuter et al. 1986; Martínez-Laborde 1988). The other (paternal) parent for *D. muralis* is considered to be *D. tenuifolia* (Harberd and McArthur 1972). Preliminary nuclear ribosomal DNA studies (S. I. Warwick and L. D. Black, in preparation) suggest that *D. simplex* of the *D. tenuifolia*/*D. simplex* (n = 11) cytodeme is a more likely parent. This finding is consistent with morphological data, wherein *D. muralis* is very similar to *D. simplex* (Schulz 1919; Maire 1965; Martínez-Laborde 1988).

The cpDNA data tended to support (with the exception of *D. simplex*) the morphologically based subgeneric taxonomic classification of Schulz (1919). The latter placed *D. tenuifolia* and *D. harra* in Sect. *Catocarpum* DC. em. O. E. Schulz and *D. viminea*, *D. muralis*, and *D. simplex* in Sect. *Anocarpum* DC. The cpDNA data were less supportive of Martínez-Laborde's (1988) taxonomic treatment, which proposes the placement of *D. harra* in a separate subgenus from *D. tenuifolia*, *D. cretacea*, *D. simplex*, *D. viminea*, and *D. muralis*. The cpDNA data were inconsistent with the placement of *D. erucoides* and *D. cossoniana* within Sect. *Rhynchocarpum* (Schulz 1919), along with the remaining *Diplotaxis* taxa observed to be in the Nigra lineage. Two of the cpDNA groups showed a high similarity to species clusters recognized in *Diplotaxis* by Takahata and Hinata (1986) on the basis of numerical morphological studies; for example, *D. harra* and the *D. tenuifolia*/*D. simplex* cpDNA subgroups correspond to clusters D-3 and D-4, respectively.

Nigra lineage

The species of *Diplotaxis* did not form a monophyletic group in the lineage, but were separated into three major groups (Groups D, E, and F) (Table 1, Fig. 1). Group D included *D. siettiana*, *D. ibicensis*, *D. brevisiliqua*, and *D. gomez-campoi*. Group E included *D. brachycarpa*. Group F included three subgroups: F (i) *D. assurgens*, *D. tenuisiliqua*, an accession of *D. virgata* from southern Morocco (DVA), and *D. siifolia*; F (ii) *D. berthautii* and *D. virgata* f. *sahariensis*; and F (iii) *D. catholica*, *D. catholica* var. *rivulorum*, *D. virgata* ssp. *virgata*.

The four taxa in the *D. ibicensis*/*D. siettiana* cpDNA group (Group D), n = 8, form a continuum geographical-

ly, with each taxon occupying a narrow region in the western Mediterranean: *D. siettiana* – Alboran Island; *D. ibicensis* – Balearic Islands; *D. gomez-campoi* – Spanish mainland; and *D. brevisiliqua* – Algeria and Tunisia (Gómez-Campo 1981; Martínez-Laborde 1988). The genetic closeness of these four taxa has been confirmed in recent morphological studies by Martínez-Laborde (1988), who placed them in a separate section in the genus. *Diplotaxis siettiana* has been given separate cytodeme status, but the other three taxa were not included in the studies of Takahata and Hinata (1983). Only one mutational difference in the cpDNA genome was detected among *D. siettiana*, *D. brevisiliqua*, and *D. gomez-campoi*. The cpDNA data is consistent with subspecific rank for these taxa. Specific rank has also only recently been suggested for the latter two taxa by Martínez-Laborde (1988, 1991c). Crossing studies by Gómez-Campo (1981) indicated that limited hybrid production was possible between *D. siettiana* and *D. ibicensis*, but only if the former taxa served as the maternal parent. Hybrids were almost completely sterile. Gómez-Campo (1981) suggested that "*D. siettiana* might be seen as an extreme variation of the polymorphic *D. ibicensis* which has become fixed by geographic isolation," but they are sufficiently divergent morphologically to retain species status. The degree of cpDNA and morphological divergence, geographic separation, and reproductive isolation is therefore consistent with specific rank for *D. siettiana* and *D. ibicensis* and their placement in a single cytodeme.

The chloroplast genome of *Diplotaxis brachycarpa* (Group E) was distinct from other species of *Diplotaxis*. This taxon is also an anomaly in the genus because of its unusual fruit morphology (Schulz 1919). Data on its cytodeme status is not available.

Group F included three subgroups: (i) *D. assurgens*, *D. tenuisiliqua*, an accession of *D. virgata* from southern Morocco (DVA), and *D. siifolia*; (ii) *D. berthautii* and *D. virgata* f. *sahariensis*; and (iii) *D. catholica*, *D. catholica* var. *rivulorum*, and *D. virgata* ssp. *virgata*. In subgroup F (i), no mutational differences were detected between *D. assurgens* and *D. tenuisiliqua*, and only a single mutational difference was observed in an accession of *D. virgata* from southern Morocco (DVA). Note that the latter accession has been recognized as a new subspecies of *D. virgata* (Martínez-Laborde 1988). The three taxa above (all n = 9) and *D. siifolia* (n = 10) were sister groups in the analysis. These taxa share a unique trait in the genus: a purple spot on the top of the anther (Martínez-Laborde 1988). Geographically they form a continuum along the coast of the Iberian peninsula and Morocco (Greuter et al. 1986; Martínez-Laborde 1988).

Subgroup F (ii) included *D. virgata* f. *sahariensis* and *D. berthautii*, each with very distinct cpDNA genomes and n = 9 chromosomes. The former taxon is endemic to the interior of Algeria and Morocco and the latter to western Morocco. CpDNA data support specific rank for each and strongly suggest that they are likely distinct cytodemes. The cytodeme status of *D. berthautii* is reported in the literature as uncertain and had been provisionally included in the *D. siifolia* cytodeme by Takahata and Hinata (1983).

Subgroup F (iii) included *D. catholica* ssp. *catholica* and var. *rivulorum* and *D. virgata* ssp. *virgata*. The three taxa (all n = 9) shared a very distinct chloroplast genome, differing among each other by only four mutations. Morphologically, all three taxa are very similar, with *D. catholica* ssp. *catholica* distinguished primarily by the presence of hooded sepals, which is likely a single-gene trait (Schulz 1919; Maire 1965; Martínez-Laborde 1988). The distribution ranges of *D. catholica* ssp. *catholica* and *D. virgata* ssp. *virgata* are sympatric throughout Spain and Portugal, with the former taxa just extending into Morocco, while *D. catholica* var. *rivulorum* is endemic to Morocco (Tutin et al. 1964; Greuter et al. 1986; Martínez-Laborde 1988).

All *Diplotaxis* taxa included in the Nigra lineage are members of Sect. *Rhynchocarpum* Prantl. em. O. E. Schulz, with the exception of *D. erucoides* and *D. cossoniana*, as indicated previously (Schulz 1919), which is equivalent to subgenus *Rhynchocarpum* Sect. *Rhynchocarpum* proposed by Martínez-Laborde (1988). The molecular data was also consistent with the separate sectional recognition proposed by Martínez-Laborde (1988) for: (i) the *D. ibicensis*/*D. siettiana* and (ii) the *D. erucoides* and *D. cossoniana* cpDNA groups. The *D. assurgens*/*D. siifolia* cpDNA group was similar to cluster D-1 recognized in numerical morphological studies of the subtribe by Takahata and Hinata (1986).

Taxonomy of subtribe Brassicinae

The placement of *Diplotaxis* species in the two distinct lineages of the subtribe indicates a polyphyletic status for the genus as currently circumscribed, similar to that described for *Brassica* and *Sinapis* (Warwick and Black 1991). Further polyphyly for the genus was indicated by separation of taxa into three major groups in each of the two lineages. Similar to the results suggested from numerical taxonomic studies of the subtribe by Takahata and Hinata (1986), these cpDNA groups of *Diplotaxis* species often showed greater genetic closeness to taxa in other genera (Fig. 1).

In the Rapa/Oleracea lineage, *D. erucoides* and *D. cossoniana* (Group A) were advanced in the lineage and very closely related to *Brassica rapa* and *B. oleracea* with its wild relatives. The genetic closeness of *D. erucoides* to *B. oleracea* had been indicated by hybrid formation and meiotic analyses (Mizushima 1980). The *D. tenuifolia*/*D. simplex* and *D. harra* cpDNA groups

(Group B) formed a separate distinct group in the lineage. *Diplotaxis viminea* and *D. muralis* (Group C) were most closely aligned to *B. gravinae*. *Eruca sativa* remained a distinct group in this lineage.

In the Nigra lineage, the *D. ibicensis*/*D. siettiana* cpDNA group (Group D) was most closely related to *Brassica nigra*, *B. fruticulosa*, *Sinapis arvensis*, and *S. alba*. *Diplotaxis brachycarpa* (Group E) was most closely related to a group of taxa that included *Sinapis pubescens*, *Hirschfeldia incana*, and *B. tournefortii*. Both *Diplotaxis brachycarpa* and *Sinapis pubescens* have n = 9 chromosomes and their distribution ranges overlap in Algeria, where the latter is endemic (Schulz 1919; Greuter et al. 1986). Group F (*D. assurgens*, *D. catholica*, *D. siifolia*) exhibited a close genetic relationship with *Erucastrum canariense* (n = 9), an endemic to the Canary Islands. The proposed relationship of the Macaronesian taxa of *Erucastrum* and adjacent continental taxa of *Diplotaxis* is of interest, as a genetic closeness between these groups has been suggested from numerical, morphologically based studies of Takahata and Hinata (1986), who indicated that "although belonging to different genera, their relationship was as close as if they were within the same genus." Gómez-Campo (1984) also reported on the taxonomic confusion often arising between these taxa as the result of their similarity in vegetative and floral characters. *Coincya* formed a distinct group in the Nigra lineage.

Taxonomic realignment at both the generic and subtribal levels that would more accurately reflect genetic relationships was discussed in Warwick and Black (1991). The molecular data presented in this paper are consistent with the expansion of the genus *Brassica* to include related genera in the subtribe, recognition of the two lineages as subgenera and major groups of taxa within each lineage as sections. Percent cpDNA divergence across the two lineages is ca. 3%, and is consistent with values for other genera (Palmer 1987). Further studies are in progress to test the placement of remaining genera and cytodemes in the subtribe, a requirement before its taxonomic revision is undertaken.

In conclusion, the restriction site variation in the chloroplast genome of *Diplotaxis* is consistent with the reported high congruence between the cpDNA taxon groups and recognized cytodemes or crossing groups in subtribe Brassicinae (Warwick and Black 1991), and with phytogeography of the genus. Chloroplast DNA data are of value in delimiting cytodemes (nuclear genomic relationships), detecting potential new breeding material, evaluating wild germplasm collections, and prioritizing future collecting needs.

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Appendix

Chloroplast DNA restriction site and length mutations in the genus *Diplotaxis* for each of 17 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. Taxa codes for *Diplotaxis* are given in Table 1. Symbols DAT (= DAS, DTT), DC (= DC1, 2), DEE (= DEE1, 2), DH (= DHH, DHC, DHG, DHF), DIS (= DIB, DST, DBV, DGC), DS (= DSS, DSB), DT (= DT1, 2), DVM (= DV, DM), unless otherwise indicated. All mutations are defined relative to clones (Cl.) and restriction maps given in Warwick and Black (1991). Length mutations (insertion = Ins.; deletion = Del.) are given for only one enzyme. Taxa codes for other species (Warwick and Black 1991) include: BB (*B. barrelieri*), BC (*B. rapa*), BD (*B. deflexa*), BF (*B. fruticulosa*), BG (*B. gravinae*), BN (*B. nigra*), BO (*B. oleracea*), BX (*B. oxyrrhina*), BRV (*B. rupestris-villosa* complex), BT (*B. tournefortii*), CC (*Coincya monensis*), ES (*Eruca sativa*), EC (*Erucastrum canariense*), HI (*Hirschfeldia incana*), RRS (*Raphanus sativus* and *R. raphanistrum*), SA (*S. alba*), SO (*S. aucheri*), SP (*S. pubescens*), SR (*S. arvensis*), RE (*Reboudia microcarpa*)

Site	Cl.	Mutation	Taxa
*Bam*HI-			
31	12	10.6 + 6.2 − 16.8	DS
–	14	Ins. 0.4: 4.6 − 5.0	DEE, DCS
38	18	1.3 (1.5) + 1.8 − 3.1 (3.3)	BF, DT, DC, DSX
39	18	1.8 + 2.8 [5.2] − 4.6 (7.0)	BN, DEE
40	18	2.8 [4.6] + 2.6 − 5.4 (7.0)	SR, BT, DEE
–	18	Del. 0.2: 2.6 − 2.4	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, ES, DT, DC, DSX, DVM, DH, DEE, DCS
*Bam*HI-			
42	16	1.2 + 1.2 − 2.4	DIS
47	19	9.2 − 5.9 + 3.3	SR, BN, DBR
47A	19	9.2 − 8.5 + 0.7	DCA, DCR, DVV
50	19	9.2 + 2.7 (9.1) − 11.9 (18.3)	BC, DHH, DHC, DHF
51	19	9.1 − 2.7 + 6.4	BC, BO, BRV, DHG
54	20	3.4 − 2.5 + 0.9	BF, DBR
–	20	3.4 − 3.6	DH, DCS
56	21	1.2 + 4.0 − 5.2	DBR

Appendix (Continued)

Site	Cl.	Mutation	Taxa
*Bcl*I-			
22	12	2.3+4.8 (9.7)−7.1 (12.0)	SO, BC, RRS, BO, BF, BT, BB, BRV, BD, ES, EC, DT, DC, DSX, DH, DEE, DCS, DAT, DVA, DS
23	12	9.7−4.8+4.9	SA, BN, HI, EC, CC, DIS, DAT, DBR, DVS, DVA, DBE, DS
25A	14	4.3−2.2+2.1	DIS
30	18	[16.3]−4.3+12.0	EC, DAT, DVS, DVA, DS
31	18	[16.3]−14.0+2.3	SA, SR, BN, BF, DIS
32	18	2.3 [19.8]+0.5−2.8 (20.3)	DIS, DCA, DCR, DVV
33	18	0.5+1.8−2.3	SO, BC, RRS, BO, BF, BB, BRV, BX, BD, DEE, DCS
37	19	2.0+10.0−12.0	SO, BC, RRS, BO, BF, BB, BRV, BX, BD, DEE, DCS
39	19	10.0−6.5+3.5	RE, DH
42	19	5.5−1.0+4.5	DT1
43	20	5.5+[5.1]−10.6	BP, DEE, DAT, DVA
44	20	[5.1]−3.6+1.5	SP, BT, DBR
–	20	1.6−1.7	BC, BO, BRV, DVS
48	21	1.6+1.4−3.0	DEE, DCS
*Bgl*I-			
3A	10	20.0−9.7+10.3	DH
3B	10	[27.2]−13.3+13.9	DCS
4	10	[27.2]−20.0+7.2	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, ES, DT, DC, DSX, DVM, DH
5A	11	42.0−20.0+22.0	DH
*Bgl*II-			
27	12	0.8+5.1−5.9	SP, BT, BD, CC, DBR
28	12	5.1−3.3+1.8	EC, DAT, DVA, DS
30	12	3.0−2.8	BB, BG, ES, DVM
36	14	7.0−5.0+2.0	EC, DAT, DVA, DS
37A	14	9.0−1.0+8.0	DBE
42	18	4.6+2.2−6.8	DEE2
43	18	2.2+[3.0]−5.2	BT, EC, DAT, DVA, DS
44	18	2.0+1.0−3.0	SP, SO, BT, EC, DVM, DAT, DVA, DS
–	19	Del. 0.3: 4.3−4.0	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, ES, DT, DC, DSX, DVM, DH, DEE, DCS
46	19	4.0+2.4−6.4	ES, DH
52	19	0.8+0.7−1.5	BT, DVS, DBE
55	19	1.0+2.7−3.7	SO, BC, RRS, BO, BB, BRV, BX, BD, RE, ES, CC, DT, DC, DSX, DVM, DH, DEE, DCS
*Bst*EII-			
11	12	7.5+9.2−16.7	ES, DH
12A	14	5.0−2.1+2.9	DS
13	14	5.3+18.0−23.3	DIB

Appendix (Continued)

Site	Cl.	Mutation	Taxa
*Cla*I-			
25	10	10.7−7.2+3.5	SO, BC, RRS, BO, BRV, BD, DT1, DC, DSX, DH, DEE, DCS
27	12	2.1−1.8+0.3	SO, BC, RRS, BO, BRV, BD, EC, DT, DC, DSX, DVM, DH, DEE, DCS, DAT, DBR, DCA, DCR, DVV, DVS, DVA, DBE, DS
28	12	2.1+0.9−3.0	SP, BT, HI, DBR, DCA, DCR, DVV
29	12	0.9−0.7+0.2	CC, DVM, DH
37	14	3.3+2.5−5.8	BC, BO, BRV, DEE, DCS, DBR
38	14	2.5−1.6+0.9	BT, DT, DC, DSX, DH
41A	14	1.2−0.7+0.5	DAT, DVA, DS
45	17	9.1−3.6+5.5	ES, DT, DC, DSX, DHH, DHC, DHG
49	18	7.0−4.7+2.3	SO, BC, RRS, BO, BB, BRV, BX, BD, ES, DT, DC, DSX, DVM, DH, DEE, DCS
49A	18	2.3−1.6+0.7	DH
50	18	7.0 [10.0]−5.7+1.3 (4.3)	BF, DVV
52	18	[5.1]+0.8−5.9	SO, BG, BRV, RE, EC, DBR
54	19	0.7+1.7−2.4	DBE
54A	19	1.7−1.3+0.4	DCA, DCR, DVV
55	19	1.7+1.7−3.4	DEE, DCS, DVS
–	19	1.7, 1.7−1.8, 1.6	DVM
56	19	1.7+2.2−3.9	SR, BN, BF, BT, BX, HI, DIS
56A	19	[8.2]−1.2+7.0	DVS
57	19	2.2 [3.9]+6.0−8.2 (9.9)	SR, BT, DT, DC
57A	19	[8.2]−3.0+5.2	DAT, DVA
57B	19	6.0−4.9+1.1	DBE
58	19	6.0−3.6+2.4	DEE
59A	19	2.0−1.3+0.7	DIS
59B	19	[1.3]−1.1+0.2	DST, DBV, DGC
60	19	2.0+9.4−11.4	SP, SR, BN, BT, BRV, BD, RE, ES, EC, CC, DT, DC, DSX, DHH, DHC, DHF, DEE, DCS
63	20	4.8+2.6−7.4	SP, RRS, BT, BG, ES, DVM, DVS
66	21	2.6+1.6−4.2	DBR
67	22	1.6+0.5−2.1	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, RE, DT, DC, DSX, DVM, DH, DEE, DCS, DS
*Dra*I-			
33	10	0.5+3.2−3.7	DVM
35	10	3.2−1.5+1.7	DEE, DCS, DT2
36	10	3.2−1.9+1.3	BG, DBE
37A	10	3.2−2.4+0.8	DH
38	10	3.2−2.6+0.6	SP, HI, DBR
42	12	2.8+1.7−4.5	DIS
42A	12	1.7−1.0+0.7	DBR, DBE
43	12	1.7−1.1+0.6	SR, DEE, DCS
–	12	1.2−1.1	DCA, DCR

Appendix (Continued)

Site	Cl.	Mutation	Taxa
*Dra*I-			
45	12	1.2+2.0−3.2	SO, BC, RRS, BO, BB, BD, DT, DC, DSX, DVM, DH, DEE, DCS
45A	12	2.0−1.1+0.9	DAT, DVS, DVA, DBE, DSB
–	12	[3.2]−2.8	BC, BO, DHC
47	12	2.0+0.8−2.8	SP, DBR
48A	13	5.2−3.2+2.0	DAT, DVA
50	14	5.2+5.2−10.4	SP, BT, HI, DBR
50A	14	[2.3]−1.4+0.9	DIS
50B	14	[2.3]−2.0+0.3	DCA, DCR, DVV
52	14	5.2−2.3+2.9	BC, BO, BRV, DT, DC, DSX, DH
53	14	[2.9]−2.4+0.5	BC, DEE, DCS
54	14	[10.4]+1.2−11.6	DBR
55	14	1.2−0.7+0.5	BN, DCA, DCR, DVV, DBE, DS
59	17	8.5−2.8+5.7	SA, DVM
59A	17	8.5−3.0+5.5	DCS
60	17	8.5−3.5+5.0	DT, DC, DSX
61A	18	[3.5]−0.7+2.8	DCA, DCR, DVV
62	18	[3.5]−3.0+0.5	SA, SR, BN, BF, BG, DIS
65	18	1.6+1.7−3.3	DVM
66A	18	1.7−0.9+0.8	DCA, DCR
69	18	1.7−1.4+0.3	SP, DBR, DBE
71	18	1.4+1.8−3.2	ES, DT, DC, DSX
71A	16	[3.2]−2.0+1.2	DT2, DSX
72	19	1.8+1.1−2.9	SP, BC, BO, BT, BRV, ES, HI, DEE, DCA, DCR, DVV, DVS
74	19	0.5+4.0−4.5	DVM, DBR
74A	19	4.0−2.3+1.7	DIS
74B	19	4.0−2.5+1.5	DHH
74C	19	4.0−3.2+0.8	DCS, DCA, DCR, DVV
78	19	2.4+3.8−6.2	DST, DBV, DGC
–	19	2.4, 3.8−3.0, 3.2	DT, DC, DSX, DH
–	19	2.4, 3.8−4.1, 2.1	DCS
79	19	3.8 (3.5)−2.3 (2.0)+1.5	BX, DAT, DVA, DBE, DS
81	19	0.8+0.9−1.7	BN, DH, DAT, DVA, DBE, DS
85	20	1.1+[2.0]−3.1	DVM
86	20	3.0−2.0+1.0	SO, BC, BO, BG, BRV, BX, BD, ES, CC, DT, DC, DSX, DVM, DH, DEE, DCS
89	20	1.2−1.0	DEE, DCS
90A	20	1.9−1.0+0.9	DH, DBE
92	20	[1.6+0.7]−2.3	SO, BC, BO, RRS, BB, BG, BRV, BX, BD, ES, DT, DC, DSX, DVM, DH, DEE, DCS
93	20	2.3−1.8+0.5	BC, BO, DH
94A	20	1.5−1.2+0.3	DVS
95	21	1.5+1.8−3.3	BB, DSX
*Eco*RI-			
33	10	1.4+0.7−2.1	SO, BC, RRS, BO, BB, BRV, BX, BD, RE, ES, EC, DT, DC, DSX, DVM, DH, DEE, DCS, DST, DBV, DGC
34	10	0.7+0.6−1.3	SP, DBE
38	10	2.4 [1.4]+2.7−5.1 (4.1)	SP, BT, BD, HI, EC, DBR
38A	12	[5.0]−0.8+4.2	DCS
39	12	2.7+2.3−5.0	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, ES, HI, EC, DT, DC, DSX, DVM, DH, DEE, DCS
39A	12	2.3−1.4+0.9	DIS
–	12	2.0−1.8	SO, BC, BO, BG, BRV, ES, DT, DC, DSX, DVM, DH, DEE, DCS
–	12	1.9−2.0	DAT, DVA
49	14	0.9+0.8−1.7	SP, BO, BF, BG, BD, DBR
52	14	7.4−0.6+6.8	SA, DIS, DVS
55	17	7.4+7.5−14.9	EC, DAT, DVA, DS
–	18	2.4−2.1	SP, BC, RRS, BO, BRV, BX, ES, EC, DVM, DH, DEE, DCS, DAT, DBR, DCA, DCR, DVV, DVS, DVA, DBE, DS
64A	16	4.5−1.3+3.2	DVM, DCA, DCR, DVV
69	19	1.6+2.8 [1.3]−4.4 (2.9)	BF, BB, BX, EC, DGC, DAT, DCA, DCR, DVV, DVS, DVA, DBE, DS
69A	19	[4.4]−4.0+0.4	DVS, DBE
70	19	2.8−1.3+1.5	SO, BC, BO, RRS, BG, BRV, BD, ES, DT, DC, DSX, DVM, DEE, DCS
71	19	2.8+2.7 (5.0)−5.5 (7.8)	BN, BB, BX, DEE, DIS, DAT, DCA, DCR, DVV, DVA
72	19	5.0−0.7+4.3	BC, BO, BRV, DEE, DCS
74	19	5.0−2.7+2.3	BN, BT, CC, DBE
74A	20	2.3−2.0+0.3	DBE
–	20	0.6−0.4	DIS
78	20	1.9+1.8−3.7	HI, DT, DC, DSX
–	21	1.6−1.5	DT, DC, DSX
85	22	[1.9]−1.0+0.9	SO, BC, RRS, BO, BRV, BX, BD, RE, ES, EC, DT, DC, DSX, DH, DEE, DCS, DAT, DVA, DS
–	14	3.8−3.7	DAT, DVA, DS
–	14	1.1, 1.6−1.0, 1.4	DVM
21	14	2.3−2.0+0.3	SA, SR, BN, BF, DIS
26	14	1.1+1.6−2.7	BB, DBR
26A	14	1.6−1.0+0.6	DVS
29	18	8.6−6.4+2.2	BG, DVM

Appendix (Continued)

Site	Cl.	Mutation	Taxa
*Eco*RV-			
31A	18	2.0 (10.2)−0.5+1.5 (9.7)	DAT, DCA, DCR, DVV, DVS, DVA, DBE, DS
32	18	2.0+8.2 [8.7]−10.2 (10.7)	SA, BG, DT, DC, DSX, DEE, DCS, DIS, DAT, DVA, DS
32A	18	[8.7]−3.2+5.5	DVS
32B	18	8.2−4.3+3.9	DBE
35	19	[2.3] (10.2)+0.5−2.8 (10.7)	SP, DEE, DCS, DVS
–	19	Del. 0.2: 0.8−0.6	DVS
39A	20	11.0−2.8+8.2	DEE2
40	20	11.0−7.0+4.0	BB, DT, DC, DSX
–	20	1.3−1.1	SO, BC, RRS, BO, BB, BRV, BX, BD, ES, DEE, DCS, DH
43	20	9.0+4.5−13.5	BT, BB, BX, ES, DBR
*Hind*III-			
25	12	6.8−4.8+2.0	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, ES, DT, DC, DSX, DVM, DH, DEE, DCS
26	12	6.8+13.0−19.8	DBR
28	11	13.0+9.0−22.0	SA, SR, BN, DIS
31	18	2.8+2.6−5.4	DCS
34A	19	7.0−4.0+3.0	DIS
34B	19	7.0−5.0+2.0	DCA, DCR, DVV
36	19	3.3−2.1 (2.3)+1.2	DT, DC, DSX
–	19	2.1−2.3	DSX
–	19	Del. 0.2: 1.6−1.4	DH
–	19	Ins. 0.1: 1.6−1.7	DCA, DCR, DVV
42	20	3.0+3.5−6.5	DEE, DCS
43A	20	7.0−1.2+5.8	DSX
*Nci*I-			
32	10	4.3+2.1−6.4	DT, DC
37A	12	[2.3]−1.2+1.1	DIS
39	12	[2.3]+2.7−5.0	DAT, DCA, DCR, DVV, DVS, DVA, DBE, DS
40	12	5.4−0.5+4.9	BG, DVM, DBE
41	12	2.7+2.7−5.4	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, RE, ES, DT, DC, DSX, DVM, DH, DEE, DCS
42	14	5.4+1.9−7.3	DCA, DCR, DVV
47	14	[2.3]−1.8+0.5	SA, SR, BN, BF, RE, CC, DIS, DVS, DBE, DS
48A	14	3.7−3.2+0.5	DCA, DCR, DVV
50	11	2.7−0.5+2.2	BG, DT, DC, DSX
52	17	2.7+1.3−4.0	BF, BX, EC, DAT, DVA, DS
56	18	1.7−0.85+0.85	DT, DC, DSX, DCA, DCR, DVV, DS
58	18	3.6−2.8+0.8	BG, DEE
62A	18	2.2−1.6+0.6	DVM
64	19	1.1+1.1−2.2	DAT, DVA
68	19	14.9−2.3+12.6	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, ES, DT, DC, DSX, DVM, DH, DEE, DCS

Appendix (Continued)

Site	Cl.	Mutation	Taxa
*Nci*I-			
70	19	[12.6]−3.2+9.4	RRS, BT, BB, BG, BRV, BX, ES, DT, DC, DSX, DVM, DH, DEE, DCS
72	19	9.4−5.4+4.0	DT, DC, DSX
72A	19	9.4−7.7+1.7	DCS
73A	20	5.0−2.7+2.3	DSS
–	20	Ins. 0.4: 5.0−5.4	DT, DC, DSX
75	20	1.6+0.4−2.0	RE, EC, DAT, DVS, DVA, DBE, DS
*Pst*I-			
6A	10	13.0−9.5+3.5	DS
7	12	13.0+2.6−15.6	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, RE, ES, DT, DC, DSX, DVM, DH, DEE, DCS, DBE
8	12	2.6+16.6−19.2	BB, BG, CC, DBE
12	17	1.05+16.5−17.6	DH, DCA, DCR, DVV
14	21	[30.4]−24.4+6.0	ES, DH, DCS
15	21	[30.4]−28.4+2.0	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, DT, DC, DSX, DVM, DH, DEE, DCS
16	21	2.0+1.6−3.6	DVM
*Sac*I-			
5A	10	[3.0]−1.8+1.2	DIB
5B	10	5.5−3.0+2.5	DIS, DAT, DCA, DCR, DVV, DVS, DVA, DBR, DBE, DS
6	12	5.5+9.0−14.5	DCS
12	18	11.5+1.05−12.6	DS
15	19	15.0−5.0+10.0	DT, DC, DSX
15A	19	15.0−11.8+3.2	DH
*Sca*I			
11	10	13.0−2.0+11.0	BG, BD, DT2, DVM
14	14	4.3+5.0−9.3	DVM
18	14	1.0+4.6−5.6	RRS, BT, BG, DT, DC, DSX, DBR
19A	18	14.0−9.6+4.4	DH
22	19	2.2+9.0−11.2	SR, BN, DAT, DVA, DS
23	19	9.0−3.8+5.2	BG, DVM
25	19	1.2+3.0−4.2	DT, DC, DSX, DBE
26A	19	14.0−2.1+11.9	DHC, DHG, DHF
26B	19	14.0−2.8+11.2	DVM
26C	19	14.0−7.0+7.0	DAT, DVA, DS
*Stu*I-			
10A	12	6.5−3.5+3.0	DCA, DCR, DVV
11	13	6.5+15.0−21.5	SO, BC, RRS, BO, BB, BG, BRV, BX, BD, ES, DT, DC, DSX, DVM, DH, DEE, DCS
15	18	6.6−3.0+3.6	DEE
16	19	6.6+6.5−13.1	DCA, DCR, DVV
19	20	8.5+4.3−12.8	BB, BG, DVM, DAT
22	21	1.4+9.0−10.4	BC, BO, RE, DCA

Appendix (Continued)

Site	Cl.	Mutation	Taxa
*Xba*I-			
17	10	2.6+4.1−6.7	DT, DC, DSX
18A	10	1.5−0.9+0.6	DSB
19A	12	6.7−0.5+6.2	DIS
23	12	6.5+0.9−7.4	BG, DT1, DC, DVM
24	14	0.9−0.5+0.4	SP, DCA, DCR, DVV
27	14	2.4+16.4−18.8	DBR, DBE
28	14	16.4−2.8+13.6	DEE, DCS
28A	14	16.4−3.6+12.8	DVM
32A	18	[2.6]−1.7+0.9	DVS
33	18	4.3−2.6+1.7	SA, SR, BN, DIS, DAT, DCA, DCR, DVV, DVS, DVA, DBE, DS
33A	18	[1.7]−1.4+0.3	DCA, DCR, DVV, DS
35	18	(4.1)+1.0−5.1	BG, RE, DVM, DEE, DCS, DBE
35A	18	[1.0+1.7]−2.2+0.5	DBE
38	19	1.1+4.0−5.1	DBR, DBE
40	19	4.0+7.0−11.0	DCS
40A	19	7.0−5.6+1.4	DVS
40B	19	7.0−5.8+1.2	DCA, DCR, DVV
40C	19	7.0−5.9+1.1	DSX
43	20	[4.7]−2.2+2.5	BC, BO, DEE, DCS
45	20	1.8−1.1+0.7	SO, BC, RRS, BO, BRV, BX, DT, DC, DSX, DVM, DH, DEE, DCS, DAT, DCA, DCR, DVV, DVS, DVA, DBE, DS
–	20	1.8, 1.6−1.9, 1.5	SO, BC, RRS, BO, BB, BRV, BX, ES, DT, DC, DSX, DH, DEE, DCS
49	20	1.7−0.9+0.8	SO, BC, RRS, BO, BB, BRV, BX, ES, DT, DC, DSX, DH, DEE, DCS
50	20	1.7+[3.5]−5.2	HI, DBE
51	20	[3.4]+[1.8]−5.2	HI, DHH, DAT, DCA, DCR, DVV, DVA, DS
53	20	0.6+3.7−4.3	SA, SR, BN, BF, DIS
*Xho*I-			
8	10	4.1+5.8−9.9	DT, DC, DSX, DBE
11	14	9.8−5.8+4.0	BG, DT, DC2, DVM
13	14	9.8−9.0+0.8	BB, DVS
14	14	[4.0]+11.1−15.1	DT, DC, DSX
16	18	11.1+20.0−31.1	DVS, DBE
17	19	20.0+15.0−35.0	EC, DAT, DVA, DS
20	20	2.2+6.5−8.7	SO, BC, RRS, BO, BB, BRV, BX, BD, CC, DEE, DCS
21	21	6.5−2.8+3.7	EC, DAT, DVA, DS

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