MOLECULAR RELATIONSHIPS OF BRASSICA AND ALLIED GENERA

S.I. Warwick

Biosystematics Research Centre, Agriculture Canada, Central Experimental Farm, Ottawa, ON CANADA K1A 0C6

INTRODUCTION

The importance of both chloroplast DNA (cpDNA) (Palmer et al. 1983; Yanagino et al. 1987) and nuclear DNA (Song et al. 1988; 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 (eg. 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. The latter includes nine genera (Brassica, Coincya, Diplotaxis, Eruca, Erucastrum, Hirschfeldia, Sinapidendron, Sinapis, and Trachystoma (Gómez-Campo 1980). Taxonomic confusion and nomenclatural synonymy across genera in the subtribe is common. On the basis of chromosome number and crossing ability, Harberd (1976) defined the Brassica "coenospecies" as the taxa capable of experimental hybridization with cultivated Brassicas. 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. 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 (Prakash and Hinata 1980). A more natural classification 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.

Chloroplast DNA restriction site variation will be used to test taxonomic classifications and species and cytodeme relationships among and within Brassica and allied genera; and to assess the correlation between cpDNA data, cytodeme status, chromosome number, and primitiveness in the subtribe.

MATERIALS AND METHODS

Seeds of each taxa (see Fig. 1) were obtained from gene banks (primarily Gómez-Campo Collection, E.T.S.I.A.). Three to six plants of each accession were grown in a greenhouse. Voucher specimens of each accession were verified and deposited in the Vascular Plant Herbarium, Agriculture Canada, Ottawa (DAO). Chrmosome numbers (Fig. 1) are from Gómez-Campo and Hinata (1980).

Procedural methods for total cellular DNA extraction, purification, restriction enzyme digestion, filter hybridization, radioactive probing and autoradiography are as given in Warwick and Black (1991). DNA was digested with each of 20 restriction endonucleases and filters were sequentially probed with 25 clones from the chloroplast genome of B.

juncea (original clones were provided by J. Palmer, University Indiana). Restriction site maps of the entire chloroplast genome for 20 endonucleases, relative to clones of the B. juncea chloroplast genome, are given in Warwick and Black (1991). The c. 800 mapped restriction sites represent 4800 nucleotide bases or 3.2% of the chloroplast genome. A total of 419 mutations were observed, including both site (i.e. gain/loss) and fragment length (i.e. insertions or deletions). Data available from the author upon request.

In the phylogenetic analyses, each mutation was treated as a two-state variable. Analysis of species relationships was conducted using the computer program "Phylogenetic Analysis Using Wagner Parsimony" (PAUP) version 2.4. The shortest phylogenetic tree(s) were calculated on the basis of all mutations which were shared by two or more taxa. Alternative methods to root the tree were examined; all procedures yielded the same two lineages. Results are shown using mid-point rooting, including Reboudia microcarpa, originally placed in the Brassicinae, with current position in Tribe Brassiceae unclear (Gómez-Campo 1980).

RESULTS AND DISCUSSION

No cpDNA site variation was found among plants in an accession and very low levels of variation, i.e. 0 -.01%, within a given species. In the taxa surveyed, 221 (53%) of the 419 mutations showed variation at the interspecific level. Several cytoplasmic markers of potential use in breeding programs were identified for each taxa.

Phylogenetic analyses of the cpDNA data indicated a clear division of the subtribe into two separate evolutionary lineages (Fig. 1). 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 Brassicas into two lineages (B. nigra versus B. rapa and B. oleracea) had been suggested from earlier cpDNA data (Palmer et al. 1983; Yanagino et al. 1987) and nuclear RFLP data (Song et al. 1988; 1990) and is supported by chromosome pairing, isozyme, and morphometric data (reviewed in Prakash and Hinata 1980; Song et al. 1988). The data suggest that the two lineages diverged early in the evolution of the Brassicinae, and prior to the evolution of distinct cytodemes in the subtribe. The cpDNA data does not support the proposal (Prakash and Hinata 1980) of an ascending aneuploid series for the subtribe (common ancestor with $n = 6$ (7)). Both lineages contained species with $n = 7$ chromosomes, suggesting separate evolutionary pathways, and the absence of $n = 7$ species at the basal position in the tree.

Rapa/Oleracea Lineage

The cpDNA data agrees with nuclear genomic data (Song et al. 1988, 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. The cpDNA data and nuclear DNA studies (Song et al. 1988) support the subspecific rank of B. alboglabra (Snogerup et al. 1990). The closest group to the rapa/oleracea genome was the wild species of the B. rupestris-villosa (n = 9) complex, which formed a distinct subgroup. All taxa in this complex are morphologically similar (Snogerup et al. 1990), interfertile with B. oleracea and are members of the B. oleracea cytodeme (Harberd 1976). The low levels of cpDNA variation and morphological divergence among taxa within this complex are more consistent with subspecific recognition rather than species rank. The cpDNA data from B. cretica and B. insularis (Warwick and Black, in prep) indicate a close relationship with other members of the B. oleracea cytodeme/cpDNA cluster.

Brassica deflexa, Diplotaxis erucoides, and Sinapis aucheri, all n = 7, were closely related to each other, advanced in the lineage, and 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 each of the five n = 7 species studied (B. deflexa, D. erucoides, Erucastrum varium, S. aucheri and H. incana) represented a distinct morphological group.

Brassica oxyrrhina, treated as a subspecies of B. barrelieri in Flora Europea, are recognized as separate cytodemes (Takahata and Hinata 1983). The cpDNA data support species rank and separate specific cytodeme status of each taxa. 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. Studies in progress (Warwick et al., in prep.) suggest that other taxa in this lineage include for Diplotaxis, D. harra (n = 13), D. viminea (n = 10), and D. muralis (n = 21) and several Erucastrum spp., including: E. leucanthemum (n = 8) and E. nasturiifolium (n = 8).

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, isozyme, and nuclear DNA (reviewed in Song et al. 1988). Sinapis alba and S. flexuosa (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 1936); and is reflected in prior taxonomic treatments of H. incana = S. incana). Within S. pubescens s. lat., there were only three mutational differences, supporting subspecific ranking for the five taxa recognized in this complex. The data indicated that Sinapis was not monophyletic, and confirmed the anomalous status of S. aucheri. This had been suggested earlier on the basis of its distinct fruit morphology, chromosome number (n = 7) and endemism to

Western Iran and eastern Iraq, as compared with the Mediterranean distribution for the rest of Sinapis (Schulz 1936).

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 genus Coincya (including data from four additional taxa- Warwick and Black, in prep.) formed a distinct cpDNA group in this lineage, consistent with recent studies by Leadlay and Heywood (1990) that the genus is a single cytodeme. Erucastrum canariense was included in this lineage, and studies in progress (Warwick and Black, in prep.) indicate that other members of the genus should be included, e.g. E. littoreum (n = 8) and E. varium (n = 7). In addition, studies by Warwick et al. in prep) have indicated that several members of the genus Diplotaxis should be included in this lineage: D. assurgens (n = 9), D. catholica (n = 9), D. siettiana (n = 8), and D. siifolia (n = 10). Preliminary data (Warwick and Black, in prep) suggest that the two most primitive genera in the subtribe,, Sinapidendron and Trachystoma, also belong to this lineage. Reboudia is aligned at the base of the more primitive Nigra lineage, lending support to its inclusion in the Subtribe.

CONCLUSIONS

Phylogenetic analyses indicated a clear division of Brassica and allied genera (Subtribe Brassicinae) into two ancient evolutionary lineages: the Nigra lineage and the Rapa/Oleracea lineage. Polyphyletic origins across lineages have been observed for four of the genera studied to date, Brassica, Sinapis, Diplotaxis, and Erucastrum. Taxonomic realignments will be required at both the generic and subtribal levels in order to more accurately reflect genetic relationships. A plausible option is the expansion of the genus Brassica to include related genera, recognizing the two lineages as subgenera and cytodeme clusters as sections. Percent divergence across the two lineages based on the cpDNA data is c. 3%, which is consistent with values for other genera. In the Nigra lineage, Brassica nigra was most closely related to Sinapis arvensis and S. alba. Species with n = 7 chromosomes exist in both lineages. Hirschfeldia incana (n = 7), in the Nigra lineage was most closely related to S. 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 the closest apparent relatives (particularly D. erucoides) to B. rapa, B. oleracea and its wild relatives.

Very low levels of genetic divergence were evident among taxa within a cytodeme. A high level of congruence was observed between recognized cytodemes or crossing groups in the subtribe and the clusters defined by the cpDNA data, but provided evidence for inconsistencies in the current generic delimitations based on morphology. This correlation is significant because of the potential predictive value of cpDNA data in delimiting cytodemes and/or detecting potentially new breeding material.

REFERENCES

GOMEZ-CAMPO, C. 1980. Morphology and morphotaxonomy of the Tribe Brassiceae. In: Brassica Crops and Wild Allies. S. Tsunoda, K. Hinata and C. Gómez-Campo (eds). Jpn. Sci. Soc. Press, Tokyo. pp. 3-31.

GOMEZ-CAMPO, C. and HINATA, K. 1980. A check list of chromosome numbers in the Tribe Brassiceae. In: Brassica Crops and Wild Allies. S. Tsunoda, K. Hinata and C. Gómez-Campo (eds). Jpn. Sci. Soc. Press, Tokyo. pp. 51-63.

HARBERD, D.J. 1976. Cytotaxonomic studies of Brassica and related genera. In: The Biology and Chemistry of the Cruciferae. J.G. Vaughan, A.J. MacLeod and B.M.G. Jones (eds). Academic Press, London. pp. 47-68.

HOSAKA, K., KIANIAN, S.F., MCGRATH, J.M. and QUIROS, C.F. 1990. Development and chromosomal localization of genome-specific DNA markers of Brassica and the evolution of amphidiploids and n = 9 diploid species. Genome 33: 131-142.

LEADLAY, E.A. and HEYWOOD, V.H. 1990. The biology and systematics of the genus Coincya Porta & Rigo ex Rouy (Cruciferae). Bot. J. Linn. Soc. 102: 313-398.

PALMER, J.D., SHIELDS, C.R., COHEN, D.B. and ORTON, T.J. 1983. Chloroplast DNA evolution and the origin of amphiploid Brassica species. Theor. Appl. Genet. 65: 181-189.

PRAKASH, S. and HINATA, K. 1980. Taxonomy, cytogenetics and origin of crop Brassica, a review. Opera Bot. 55: 1-57.

SCHULZ, O.E. 1936. Cruciferae. In: Die Natürlichen Pflanzenfamilien, 2nd Ed, A. Engler and K. Prantl (eds). Engelmann, Leipzig. 17: 227-658.

SNOGERUP, S., GUSTAFSSON, M. and VON BOTHMER, R. 1990. Brassica sect. Brassica (Brassicaceae) I. Taxonomy and variation. Willdenowia 19: 271-365.

SONG, K.M., OSBORN, T.C. and WILLIAMS, P.H. 1988. Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). I Genome evolution of diploid and amphidiploid species. Theor. Appl. Genet. 75: 784-794.

SONG, K.M., OSBORN, T.C. and WILLIAMS, P.H. 1990. Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 3. Genome relationships in Brassica and related genera and the origin of B. oleracea and B. rapa (syn. campestris). Theor. Appl. Genet. 79: 497-506.

WARWICK, S.I. and BLACK, L.D. 1991. Molecular systematics of Brassica and allied genera (Subtribe Brassicinae, Brassiceae)- Chloroplast genome and cytodeme congruence. Theor. Appl. Genet. "In Press".

TAKAHATA, Y. and HINATA, K. 1983. Studies on cytodemes in subtribe Brassicinae (Cruciferae). Tohoku J. Agr. Res. 33: 111-124.

TAKAHATA, Y. and HINATA, K. 1986. A consideration of the species relationships in subtribe Brassicinae (Cruciferae) in view of cluster analysis of morphological characters. Pl. Sp. Biol. 1: 79-88.

YANAGINO, T., TAKAHATA, Y. and HINATA, K. 1987. Chloroplast DNA variations among diploid species in Brassica and allied genera. Jap. J. Genet. 62: 119-125.

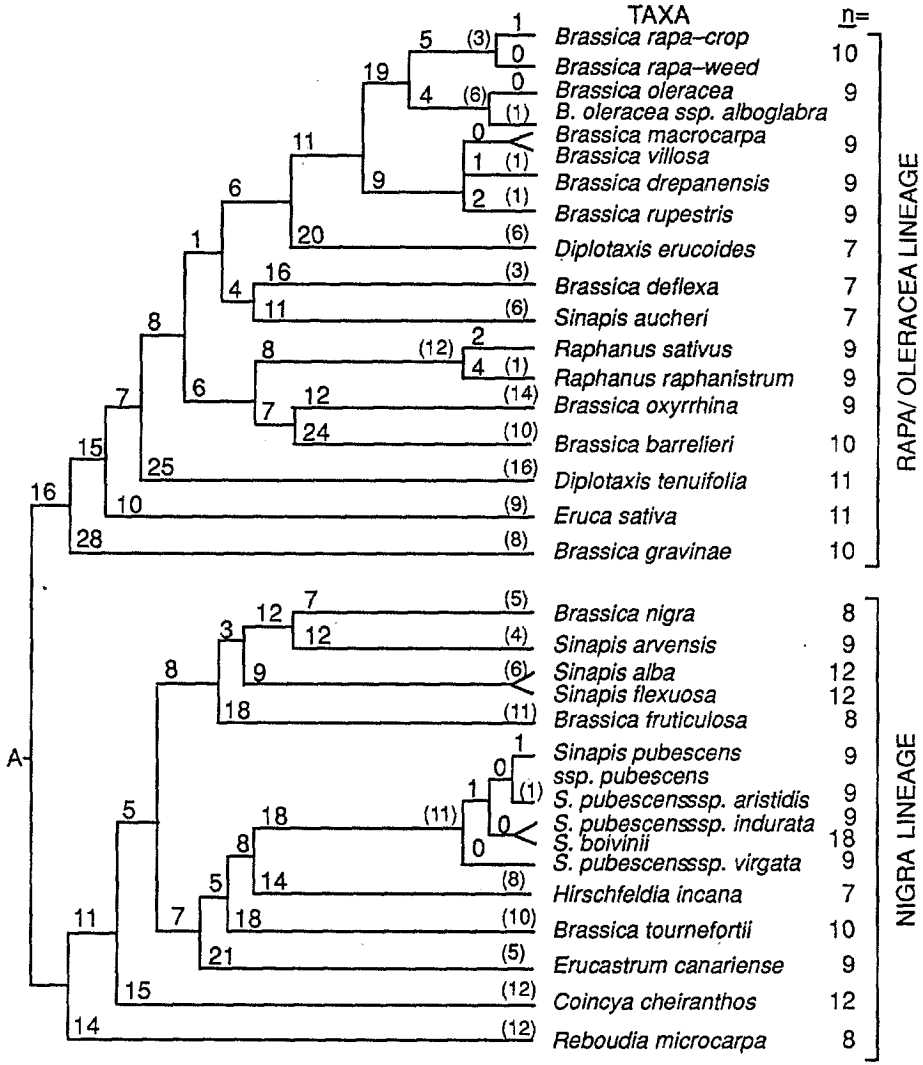

Fig. 1. Phylogenetic tree for *Brassica* and allied genera based on PAUP analyses of the chloroplast DNA restriction site mutations shared by two or more taxa. Tree length is 489 steps, consistency index 0.491. Branch length (no. above the branches), mutations unique to a given taxa (no. in brackets at end of branch), and A– shows hypothetical common ancestor.