Theor Appl Genet (1988) 76:593–600

Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs)

2. Preliminary analysis of subspecies within *B. rapa* (syn. *campestris*) and *B. oleracea*

K. M. Song [1,2], T. C. Osborn [2] and P. H. Williams [1]

[1] Department of Plant Pathology, 1630 Linden Drive, University of Wisconsin, Madison, WI 53706, USA
[2] Department of Agronomy, 1575 Linden Drive, University of Wisconsin, Madison, WI 53706, USA

Received April 15, 1988; Accepted May 3, 1988
Communicated by G. Wenzel

Summary. Preliminary analysis using nuclear RFLPs provided evidence that subspecies within *Brassica rapa* originated from two different centers. One center is in Europe, represented by turnip and turnip rape from which the oilseed sarson was derived. A second center is in South China containing a variety of Chinese vegetables of which pak choi and narinosa seem to be the most ancient forms. Based on RFLP data, the accessions of *B. oleracea* examined could be divided into three distinct groups, represented by thousand head kale, broccoli and cabbage. Thousand head kale and Chinese kale appear to be the primitive types. Observations of parallel variation among subspecies of both species are discussed.

Key words: *Brassica rapa* – *Brassica oleracea* – Molecular taxonomy – Phylogenetic tree – Restriction fragment length polymorphism

Introduction

The genus *Brassica* includes several agriculturally important species with interesting evolutionary relationships. In a previous study (Song et al. 1988), we used nuclear restriction fragment length polymorphisms (RFLPs) to examine the taxonomy and evolution of *Brassica* species. Based on RFLP data and results from other studies, we hypothesized that the three cultivated diploid *Brassica* species evolved in two pathways. One pathway gave rise to *B. nigra* with *Sinapis arvensis* as a possible progenitor. A second pathway resulted in the cultivated forms of *B. rapa* (syn. *campestris*) and *B. oleracea*.

Within *B. rapa* and *B. oleracea* there is considerable morphological variation. This variability is important agriculturally and has attracted the attention of taxonomists who are interested in phylogenetic relationships of different morphotypes. Previous studies based mainly on comparative morphology, isozymes and seed proteins suggested that the cultivated forms in *B. rapa* can be divided into two subgroups: one includes turnip and oil seed types distributed throughout Europe, Central Asia and India, and the other includes leafy types distributed in East Asia (Prakash and Hinata 1980; Vaughan 1977). Various schemes have been proposed for categorizing different morphotypes within *B. oleracea* (Prakash and Hinata 1980). Heim (1963) suggested that the cultivars in *B. oleracea* can be divided into three groups represented by thousand head kale (var. *ramosa*), broccoli (var. *italica*) and kale (convar. *acephala*). However, many aspects of the phylogeny of subspecies within *B. rapa* and *B. oleracea* are not fully understood. In particular, there is no nuclear DNA evidence for genetic divergence among different cultivated forms.

In this study, we have used RFLP data from our previous study (Song et al. 1988) to analyze more closely the possible phylogenetic relationships among morphotypes within *B. rapa* and *B. oleracea*. Our results confirm conclusions from previous studies that used different taxonomic criteria and provide new insight into the genetic diversity among subspecies at the DNA level. Also, our data point to directions for future studies in taxonomy of the *Brassica* genus.

Materials and methods

Seventeen accessions from *B. rapa* and 18 accessions from *B. oleracea* were selected as representatives of various morphotypes within these species. *B. fruticulosa* and *B. adpressa* were

Table 1. Plant materials used in the experiment and their sources

Abbr.	Subspecies	Cultivar	Source[a]
B. rapa (syn. *campestris*)			
A1	chinensis	Flowering pakchoi	CrGC
A2	pekinensis	Wong Bok	WGB
A3	pekinensis	WR 70 days	UCD
A4	pekinensis	Michihili	Olds
A5	pekinensis	Chi Hi Li	WGB
A6	japonica	Mizuna	CrGC
A7	narinosa		CrGC
A8	perviridis	Tendergreen	Olds
A10		Broccoletto	WGB
A11		Spring broccoli raab	CrGC
A13	utilis	Hong Tsai Tai	CrGC
A14	parachinensis	Choi sum	WGB
A15	rapifera	Presto	Sakata
A16	rapifera	Purple Top White Globe	CrGC
A17	trilocularis	R500, High GS	CrGC
A18	oleifera	Trunip rape	UCD
A19	trilocularis	Sarson	UCD
B. oleracea			
C1	gongylodes	Kohlrabi	Olds
C2	sabauda	Savoy cabbage	WGB
C3	botrytis	Cauliflower (All Year Round)	WGB
C4	botrytis	Cauliflower (White Rock)	CrGC
C5	gemmifera	Brussels sprout (Eveshum Giant)	WGB
C6	italica	Broccoli (Nine Star Perennial)	WGB
C7	italica	Broccoli (Packman)	Olds
C8	italica	Broccoli (Green Sprouting)	Olds
C9	palmifolia	Jersey kale	WGB
C10	medullosa	Marrow stem	WGB
C11	selensia	Borecole (Vates, Curled)	Olds
C12	alboglabra	Chinese kale (Large leaf kailan)	WGB
C13	ramosa	Thousand head kale (Dwarf)	WGB
C14	ramosa	Thousand head kale	WGB
C15	costata	Portugese cabbage	CrGC
C16	sabellica	Collards (Georgia)	Olds
C17	capitata	Cabbage (Wis. Golden Acre)	CrGC
C18	capitata	Cabbage (Brunswick)	CrGC
Brassica adpressa			
B.a.[b]			CrGC
Brassica fruticulosa			
B.f.[b]			CrGC

[a] WGB = Wellesbourne Gene Bank in England, UCD = University of California at Davis, Olds = Olds Seeds Co., CrGC = Crucifer Genetics Cooperative
[b] Outgroups

selected as outgroups for the phylogenetic analysis since these wild species are very distant from *B. rapa* and *B. oleracea* (Song et al. 1988). Accessions used in the study and their sources are listed in Table 1.

The methods used for detection of nuclear RFLPs in selected accessions were described previously (Osborn et al. 1987). Phylogenetic analyses were based on the same 20 probe-enzyme combinations (Table 2) and the same methods were used for data analysis as described previously (Song et al. 1988). In addition to phylogenetic analysis using parsimony (PAUP), we compared accessions in a pairwise fashion (Fitch and Margoliash 1967) using the total number of polymorphic restriction fragments between pairs of accessions.

Results and discussion

Information from different types of analyses

Initially, accessions were analyzed by comparing RFLPs detected by specific probe-enzyme combinations (PECs). Considerable variation in RFLP patterns were found among accessions from both species. Each PEC usually separated the accessions within a species into several groups according to similarities in RFLP pattern; however, some probes distinguished a particular group of ac-

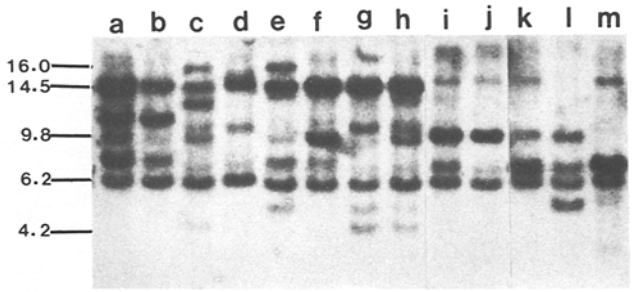

Fig. 1

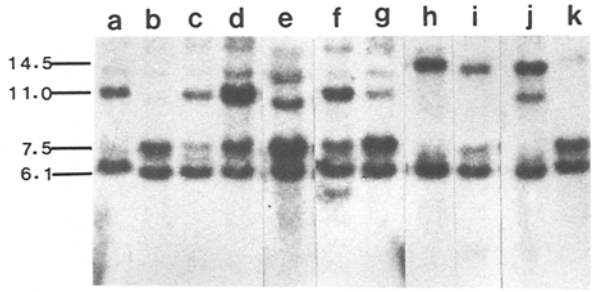

Fig. 2

Fig. 1. Autoradiographs of *Eco*RV digested DNAs probed with pC1 showing variation among subspecies. The letters indicate individual RFLP patterns and the sizes of major fragments are given in kilobases. *a* The RFLP pattern of accession C13; *b* C14; *c* C12; *d* C1, C2, C11 and C16–C18; *e* C5 and C10; *f* C9; *g* C3, C4, C6 and C7; *h* C8; *i* A2 and A4; *j* A5; *k* A1, A6, A8, A13 and A14; *l* A7; *m* A3, A10, A11 and A15–A19

Fig. 2. Autoradiographs of *Eco*RV digested DNAs probed with p21C8 showing variation among subspecies. The letters indicate individual RFLP patterns and the sizes of major fragments are given in kilobases. *a* The RFLP pattern of accession C11; *b* C12; *c* C5 and C10; *d* C13 and C14; *e* C1, C2 and C15–C18; *f* C6–C8; *g* C3 and C4; *h* A2–A5; *i* A1 and A7; *j* A8, A13 and A14; *k* A6, A10, A11 and A15–A19

Table 2. Probes and enzymes used for detecting RFLPs

Probe	Probe source	Plant source	Enzyme used
pC1	Cruciferin (seed protein)	*B. napus*	*Hin*dIII, *Eco*RI and *Eco*RV
pN2	Napin (seed protein)	*B. napus*	*Eco*RV
pK4A3	Nuclear DNA	*B. oleracea* (C17)	*Hin*dIII, *Eco*RI, *Eco*RV
pK4G11	Nuclear DNA	*B. oleracea* (C17)	*Eco*RV
p21D8	Nuclear DNA	*B. rapa* (A3)	*Hin*dIII, *Eco*RI
p21E8	Nuclear DNA	*B. rapa* (A3)	*Eco*RV
p21B9	Nuclear DNA	*B. rapa* (A3)	*Eco*RV, *Hin*dIII
p21H2	Nuclear DNA	*B. rapa* (A3)	*Hin*dIII, *Eco*RV
p21G4	Nuclear DNA	*B. rapa* (A3)	*Hin*dIII, *Eco*RV
p21E5	Nuclear DNA	*B. rapa* (A3)	*Hin*dIII
p21E3	Nuclear DNA	*B. rapa* (A3)	*Eco*RV
p21C8	Nuclear DNA	*B. rapa* (A3)	*Eco*RV

cessions from all others within a species. For instance, using the pC1-*Eco*RV combination, thousand head kale (*B. oleracea*, C13 and C14) and narinosa (*B. rapa*, A7) had unique RFLP patterns that were different from other accessions in their respective species (Fig. 1). Similarly, all Chinese cabbage accessions (A2–A5) had a distinct pattern from those of other accessions using the p21C8-*Eco*RV combination (Fig. 2). Although morphologically related accessions were often found to have similar RFLP patterns, the accessions in each group varied depending on the PEC used. Therefore, it is difficult to access the phylogenetic relationships between accessions by comparing RFLP patterns for individual PECs. However, in some cases the qualitative characteristics of this comparison could be used to determine the relationship between particular accessions when analysis of data from all PECs combined did not give an unambiguous result.

The overall level of dissimilarity among accessions within species was determined by totaling the number of polymorphic restriction fragments between pairs of accessions for all 20 PECs. Values from these comparisons are presented in Table 3 for *B. rapa* and Table 4 for *B. oleracea*. The number reflects the degree of dissimilarity between pairs of accessions; in general, larger numbers represent larger differences between accessions. Thus, these data represent quantitative relationships among the taxa. In most cases, the values in Tables 3 and 4 are equal to the minimum mutation numbers on the phylogenetic trees (Figs. 3 and 4). However, a small number does not necessarily mean that the two accessions are closely related since the individual fragments are not weighted equally in terms of their phylogenetic significance, i.e., some fragments are phylogenetically more important than others. For example, the difference between A1 and A2 is 4 (Table 3), but these 2 accessions are separated into 2 distinct groups in the phylogenetic tree (Fig. 3), whereas A3 and A4, with 12 restriction fragment differences, are closely related in the same tree.

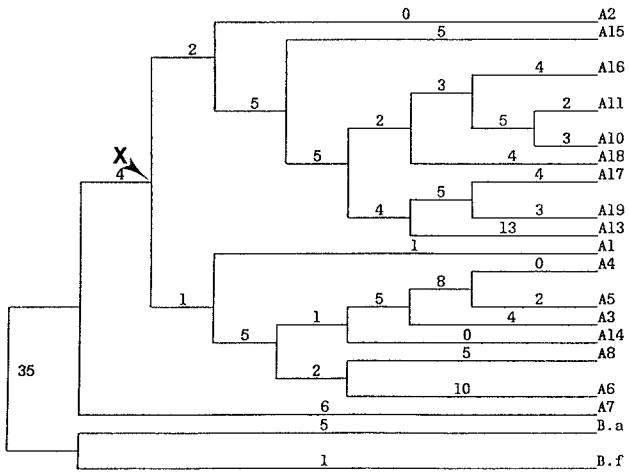

Fig. 3

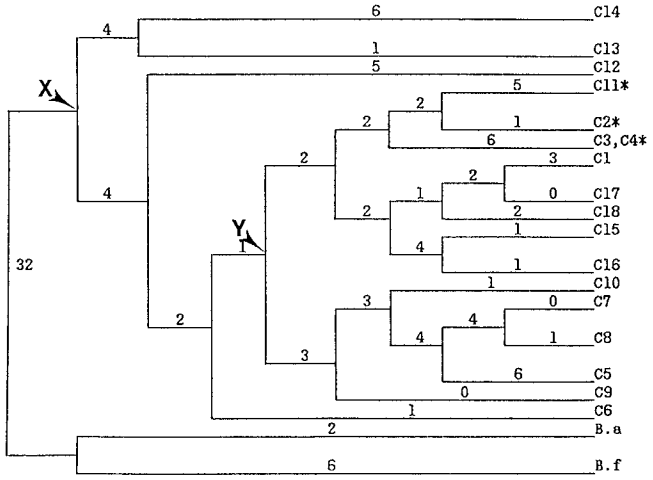

Fig. 4

Fig. 3. A selected phylogenetic tree of *Brassica rapa* (syn. *campestris*). *Letters* at the termination of branch indicate accessions (Table 1). *Numbers* on the branches indicate the minimum number of mutation steps. The point *X* (*arrow*) shows the hypothetical common ancestor of European Group and East Asian group (see text). Tree no. 2, length = 164.000, consistency index = 0.463

Fig. 4. A selected phylogenetic tree of *Brassica oleracea*. The point *X* indicates the hypothetical common ancestor where thousand head kale separated from the other accessions. The point *Y* shows the hypothetical ancestor of cabbage group and broccoli group (see text). Tree no. 21, length = 117.000, consistency index = 0.530; * positions are uncertain

Table 3. Total number of dissimilar RFLPs between pairs of *B. rapa* accessions

	A1	A2	A3	A4	A5	A6	A7	A8	A10	A11	A13	A14	A15	A16	A17	A18	A19	Ba	Bf
A1	0	4	17	13	13	20	13	12	20	24	25	7	15	20	28	19	25	53	49
A2		0	12	12	12	21	10	11	17	23	22	8	10	17	23	13	19	52	48
A3			0	12	14	19	16	13	23	29	24	10	14	23	25	22	22	59	56
A4				0	2	23	20	17	25	29	28	14	20	27	22	22	22	58	54
A5					0	25	18	19	24	28	28	16	20	27	23	22	22	56	52
A6						0	19	16	22	24	29	15	21	20	28	25	27	57	57
A7							0	9	19	25	22	12	14	17	27	20	22	52	48
A8								0	18	22	23	9	13	17	26	19	21	57	55
A10									0	8	29	21	19	12	18	13	17	57	53
A11										0	33	25	23	14	22	17	23	59	55
A13											0	24	24	25	27	26	26	66	64
A14												0	13	19	29	21	26	58	54
A15													0	11	23	16	18	56	54
A16														0	20	11	19	59	57
A17															0	15	7	60	57
A18																0	14	56	54
A19																	0	60	56
Ba																		0	18
Bf																			0

Sets of informative restriction fragments for all 20 PECs were used to construct phylogenetic trees within each species. For *B. rapa*, a total of 123 restriction fragments were recorded. There were 21 unique fragments (17.1%), 26 fragments common to all accessions (21.1%) and 76 fragments that were phylogenetically informative (61.8%) and thus used to construct a tree with the PAUP computer program (D. L. Swofford, Illinois Natural History Survey, Champaign, Ill). Using "Swap = global, Maxtree = 100" functions, the 6 shortest or most parsimonious trees were found with tree lengths of 164 and consistency indices of 0.46. These trees had similar topologies and exhibited only slight differences in the positions of accessions A1 and A14. One representative tree (Fig. 3) was selected based on the following criteria: (1) the representativeness of the overall tree topology; (2) the frequency of individual taxa at certain positions; and (3) the frequency of a particular topology of individual

Table 4. Total number of dissimilar RFLPs between pairs of *B. oleracea* accessions

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	Ba	Bf
C1	0	8	15	15	18	12	10	11	9	10	11	16	15	15	9	9	3	7	48	49
C2		0	11	11	14	6	12	13	9	12	5	12	11	17	11	11	7	5	45	47
C3			0	0	19	13	13	14	14	17	16	19	22	24	18	18	12	14	50	52
C4				0	19	13	13	13	14	17	16	19	22	24	18	18	12	14	50	52
C5					0	12	10	11	13	10	15	14	17	19	17	19	17	13	47	51
C6						0	10	11	5	8	7	8	9	15	11	11	9	5	47	49
C7							0	1	7	8	17	16	19	17	15	15	11	11	53	57
C8								0	8	9	18	15	19	15	16	16	12	12	47	49
C9									0	3	10	11	12	12	10	10	8	8	48	52
C10										0	13	10	13	11	11	13	11	11	51	55
C11											0	13	12	16	12	10	10	8	41	44
C12												0	13	15	15	17	15	11	49	45
C13													0	6	16	16	14	10	44	44
C14														0	20	20	16	16	48	48
C15															0	2	8	8	50	50
C16																0	8	8	48	48
C17																	0	4	48	48
C18																		0	46	48
Ba																			0	18
Bf																				0

groups in all possible trees. The topology of the top part of the tree as well as the topology of accessions A3–A8 at the bottom part of the three were the same in all 6 trees (100%), while the positions of A1 and A14 were the same in 4 out of 6 trees (66.7%).

For *B. oleracea*, a total of 107 restriction fragments were recorded. There were 14 unique fragments (13.8%), 29 fragments common to all accessions (27.1%) and 64 informative fragments (59.8%) that were used to construct a phylogenetic tree as described in *B. rapa*. Fifty-three trees were generated by the PAUP program using "Swap = global" and "Maxtree = 100" functions. All of the trees had a length of 117 and a consistency index of 0.53. The large number of output trees conveyed a certain amount of ambiguity in the phylogeny of *B. oleracea*. However, based on information from 50 trees, we were able to select a phylogenetic tree (Fig. 4) that represented the relationships better than any of the other trees. The criteria used in selecting this tree were similar to those for selection of the *B. rapa* tree, except that we also used the results of RFLP patterns from individual PECs and the data in Table 4 as references. In more than 40 out of 50 trees, the accessions C1, C5–C10 and C12–C18 had the same topology as that in Fig. 4, whereas the positions of C2–C4 and C11 were variable among different trees.

The same accessions and corresponding RFLP data used to construct these trees were used in a previous study to construct a phylogenetic tree for accessions from several *Brassica* species (Song et al. 1988). Although the trees in this report are very similar to the subset trees for *B. rapa* and *B. oleracea* reported previously, some differences are apparent because only the pertinent subset of accessions were used to construct the trees in Figs. 3 and 4. Details of the phylogenetic relationships within *B. rapa* and *B. oleracea* are presented below.

Phylogeny of subspecies within B. rapa

According to the phylogenetic tree in Fig. 3, the accessions within *B. rapa* can be divided into two groups. One group consisted of accessions in the top part of the tree including turnip, turnip rape and sarson. Most of these accessions originated from Europe, and thus were designated as the European group. The other group, designated as the East Asian group, consisted of accessions in the bottom part of the tree including Chinese cabbage, pak choi and other vegetable forms from East Asia. One accession, narinosa (A7), seemed to fit neither group. However, with some PECs such as P21C8-*Eco*RV (Fig. 2), this accession had RFLP patterns that were more similar to the East Asian group than to the European group.

An anomaly to these groupings is a cultivar of Chinese cabbage, Wong Bok (A2). This accession clustered with the European group, whereas other Chinese cabbage accessions (A3–A5) were assigned to the East Asian group. However, Wong Bok is distinct from all other European accessions and is only a short phylogenetic distance from pak choi, A1 (Fig. 2). Since Wong Bok is a landrace cultivar and may represent one of the primitive cultivated types of Chinese cabbage, this exception supports the hypothesis that Chinese cabbage originated from hybridization between turnip (or turnip rape) and pak choi (Li 1980).

Within the European group, turnip (A16), turnip rape (A18) and spring broccoli raab (A10, A11) are more closely related to each other than to sarson (A17 and A19). This result supports a previous hypothesis based on morphological classification that sarson came from turnip rape but had been selected and developed in India independently (Prakash and Hinata 1980). Hong Tsi Tai (A13), a bolting type endemic to China, is related to sarson and might have originated from a sarson accession introduced into China. However, the large unit distance of 13 (Fig. 3) indicates that Hong Tsi Tai has diverged considerably from sarson. The turnip accession Presto (A15), which is a highly selected cultivar from Japan, is very distinct from other accessions in the European group. Geographic isolation and intense selection pressure may be responsible for the large divergence between this accession and others.

Within the East Asian group, pak choi (A1) is likely to be the most ancient type since it is separated from the other accessions and is most closely related to the hypothetical common ancestor of both the European and East Asian groups (point *X* in Fig. 3). Pak choi, together with narinosa (A7), may represent primitive types in the East Asian group from which other forms were derived. The antiquity of pak choi is suggested also by its long history of cultivation, its vast range of morphological diversity (Li 1980) and its high level of DNA polymorphism (Figdore et al. 1988). Chinese cabbage constitutes a distinct subgroup in which the two open pollinated cultivars, Chi Hi Li (A4) and Michihili (A5), are much closer to each other than either is to the hybrid, WR 70 Days (A3). Mizuna (A6) and Tendergreen (A8) are clustered together, though there is a large divergence between them (unit distance = 15). Choi sum (A14) is a distinct form that might have been derived from one type of pak choi.

Our results agree with those of previous studies that separated *B. rapa* morphotypes into two groups (Prakash and Hinata 1980; Vaughan 1977). Based on morphology, geographic distribution, isozymes and nuclear RFLPs, these groups appear to represent two independent centers of origin. The primary center is in Europe and includes turnip and turnip rape from which sarson and toria types were derived and have evolved separately. The second center is in South China containing a variety of Chinese vegetables of which pak choi and narinosa appear to be the most ancient forms. Other forms in this group, such as *parachinensis, perividis* and *japonica*, may have been derived directly or indirectly from different varieties of pak choi. Accessions in these two centers could have evolved from separate wild *B. rapa* populations that originated in Europe and were disseminated through Central Asia into East Asia. A possible alternative evolutionary pathway is that a primitive cultivated form of *B. rapa* originating in Europe, from which the European group evolved, was introduced to South China in ancient times and subsequent selection resulted in the East Asian group of accessions. In either case, geographical isolation and climatic conditions probably played a critical role in the divergence of these two groups.

Phylogeny of subspecies within B. oleracea

Phylogenetic analysis within *B. oleracea* resulted in several clusters of accessions (Fig. 4). Two accessions of thousand head kale, C13 and C14, were separated from all other forms and were closer to the hypothetical common ancestor (point *X* in Fig. 4) than others. This suggests that thousand head kale is an ancient form of *B. oleracea* and might be the progenitor or close to the progenitor of other morphotypes. Our results agree with other studies that have proposed thousand head kale as a primitive cultivated type in *B. oleracea* (Nieuwhof 1969; Zeven et al. 1988). Chinese kale (C12) is also separated from other accessions and is relatively close to the hypothetical common ancestor (point *X*). The position of Chinese kale on the phylogenetic tree indicates that it is nearly as old as thousand head kale. Therefore, Chinese kale may have evolved from a primitive kale that was introduced from Western Europe into Eastern Asia.

The other *B. oleracea* accessions can be divided into two groups (Fig. 4). One group consisted of cabbage (C17 and C18), collard (C16), kohlrabi (C1) and Portuguese cabbage (C15) and was designated as the cabbage group. Borecole (C11) and savoy cabage (C2) appeared to be related to each other and were included in this group, although their exact placement is uncertain. Our cabbage group apparently is equal to the kale group (*acephala*) classified by morphology (Helm 1963).

The second major group within *B. oleracea* contained broccoli (C7 and C8), marrow stem (C10), Jersey kale (C9) and brussels sprouts (C5), and was designated as the broccoli group. Marrow stem and Jersey kale were close to each other. Brussels sprouts appeared to be related to broccoli, even though they were separated by a large unit distance of 12. Nine Star Perennial Broccoli (C6) was found to rank with the hypothetical common ancestor of both the cabbage and broccoli groups (point *Y* in Fig. 4), suggesting that perennial broccoli types are more primitive than annual broccoli types.

Previous studies have suggested that cauliflower originated from broccoli (Crisp 1982; Gray 1982). Our RFLP data indicate that two accessions of cauliflower (C3 and C4) are phylogenetically closer to the cabbage group than to the broccoli group, but one PEC (pC1-*Eco*RV) showed the same RFLP pattern for cauliflower and broccoli (Fig. 1). However, the large divergence of cauliflower from all other accessions (Table 4) suggests that it does not belong to either group. It is possible that cauliflower was derived from a morphotype in the cabbage group with subsequent introgression from broccoli or vice

versa, or perhaps it is of independent origin from a wild species such as *B. cretica* (Snogerus 1980). More data are needed to verify the origin of cauliflower.

Phylogenetic groupings of *B. oleracea* accessions based on nuclear RFLP data are consistent with previously proposed groupings based on morphological differences (Prakash and Hinata 1980). Both studies divided accessions into three groups represented by thousand head kale, cabbage and broccoli, although the assignment of some morphotypes to these groups differed in the two studies. Since we have examined only a small portion of the *B. oleracea* gene pool using relatively few RFLPs, our assignment of morphotypes to these groups is still preliminary.

The three groups may have all originated from a single primitive cultivated form that was distributed through Europe in ancient times. Alternatively, these groups may have originated independently in different regions of Europe from various wild forms of the *B. oleracea* group. Some evidence from the study of glucosinolates (Mithen et al. 1987) supports this latter hypothesis. Additional RFLP studies including wild species should provide more information on the origin of *B. oleracea* accessions.

Comparison of subspecies evolution in B. rapa and B. oleracea

Within both *Brassica* species, the vast array of morphological variation is accompanied by considerable variation at the DNA level. Overall, there is more DNA polymorphism among accessions of *B. rapa* than among *B. oleracea* accessions (Tables 3 and 4). This observation suggests that although *B. oleracea* is thought to be the older of the two species, there is more genetic variation within *B. rapa*, possibly due to the wider geographic distribution of this species.

Within each species there are apparent differences among accessions or groups of accessions in the rates of molecular divergence. For example, two accessions, A1 (flowering pakchoi) and A2 (Wong Bok) in Fig. 3, have a unit distance of only 2 from the hypothetical common ancestor (point *X*). They seem to have diverged much less than other accessions (unit distances of 6–29). Similarly, within *B. oleracea*, accessions C6 and C12–C14 have diverged less than the other accessions (Fig. 4). Theoretically, the unequal rates of molecular divergence could result from differential selection intensities and/or genome introgression. Higher selection pressures and/or frequent introgression may cause faster rates of change.

In a previous report we suggested that *B. rapa* and *B. oleracea* have a common ancestor in the 9 chromosome wild species (Song et al. 1988). Within each species, there are parallelisms in groupings based on both morphological and nuclear DNA variation. Based on RFLP analysis, the accessions examined cluster into morphologically similar phylogenetic groups in each species. The morphological variation in the European group of *B. rapa* is similar to that of the broccoli group in *B. oleracea*, whereas the variation in the East Asian group of *B. rapa* is similar to that of the cabbage group in *B. oleracea*. This parallelism indicates a similarity between the two genomes in the range of genetic variability, and thus gives further evidence for the common origin of these two species.

This study demonstrates the usefulness of nuclear RFLPs in detecting genetic variation among populations and provides a further understanding of phylogenetic relationships between subspecific forms of *B. rapa* and *B. oleracea*. However, ambiguities still exist concerning the phylogeny of individual accessions. Further work is needed to clarify the origins of these species and subspecies. Studies that include more wild species would be more informative in this regard. Also, where introgression between subspecies may have occurred, the use of more DNA probes that map to different genomic regions will allow more thorough analysis of evolutionary pathways.

Acknowledgements. We are grateful to Dr. K. J. Sytsma for advice on computer analysis of the data and for critical review of the manuscript, to Wellesbourne Gene Bank in England for providing most of the plant materials and to Dr. M. L. Crouch for providing the probes pC1 and pN2. Funding was provided by the Department of Agriculture of China, by the College of Agriculture and Life Science, University of Wisconsin and by the National Kraut Packers Association.

References

Crisp P (1982) The use of an evolutionary scheme for cauliflowers in the screening of genetic resources. Euphytica 31:725–734

Figdore SS, Kenard W, Song KM, Slocum MK, Osborn TC (1988) Assessment of the degree of restriction fragment length polymorphism in *Brassica*. Theor Appl Genet 75:833–840

Fitch WM, Margoliash E (1967) Construction of phylogenetic trees. Science 155:279–284

Gray AR (1982) Taxonomy and evolution of broccoli (*Brassica oleracea* var. *italica*). Econ Bot 36:397–410

Helm J (1963) Morphologisch-taxonomische Gliederung der Kultursippen von *Brassica oleracea* L. Kulturpflanze 11:92–210

Li CW (1980) The origin, evolution, taxonomy and hybridization of Chinese cabbage. In: Talekar NS, Griggs TD (eds) Chinese Cabbage. Asian Vegetable Research Center, Taiwan, RO China, pp 1–10

Mithen RF, Lewis BG, Heaney RK, Fenwick GR (1987) Glucosinolates of wild and cultivated *Brassica* species. Phytochemistry 26:1969–1973

Nieuwhof M (1969) Cole Crops. In: Polunin N (ed) Leonard Hill Book, London, pp 1–25

Osborn TC, Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor Appl Genet 73:350–356

Prakash S, Hinata K (1980) Taxonomy, cytogenetics and origin of crop *Brassica*, a review. Opera Bot 55:1–57

Snogerup S (1980) The wild forms of the *Brassica oleracea* group ($2n=18$) and their possible relations to the cultivated ones. In: Tsunoda S, Hinata K, Gomez-Campo C (eds) Brassica Crops and Wild Allies. Jpn Sci Soc Press, Tokyo, pp 121–132

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLPs). 1. Genome evolution of diploid and amphidiploid species. Theor Appl Genet 75:784–794

Vaughan JG (1977) A multidisciplinary study of the taxonomy and origin of *Brassica* crops. BioScience 27:35–40

Zeven AC, Ramanna MS, Boeder M, Sawor Z, Waninge J (1988) Diploids and natural autotetraploids in the predominantly vegetatively propagated *Brassica oleracea* L. var *ramosa* DC and their cytology. Euphytica (in press)