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E. J. R. Bohuon · D. J. Keith · I. A. P. Parkin
A. G. Sharpe · D. J. Lydiate

Alignment of the conserved C genomes of *Brassica oleracea* and *Brassica napus*

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Abstract A population of 169 microspore-derived doubled-haploid lines was produced from a highly polymorphic *Brassica oleracea* cross. A dense genetic linkage map of *B. oleracea* was then developed based on the segregation of 303 RFLP-defined loci. It is hoped that these lines will be used by other geneticists to facilitate the construction of a unified genetic map of *B. oleracea*. When the *B. oleracea* map was compared to one of *B. napus* (Parkin et al. 1995), based on the same RFLP probes (Sharpe et al. 1995), good collinearity between the C-genome linkage groups of the two species was observed.

Key words Restriction fragment length polymorphism (RFLP) · Genetic linkage · Comparative mapping · Doubled-haploid lines · Reference population

Introduction

Brassica oleracea is an important crop species with a wide range of vegetable forms including cabbage, broccoli, cauliflower, kale, kohlrabi and brussels sprouts. The recent development of molecular markers in *B. oleracea* will facilitate marker-assisted breeding in these crops. Three genetic linkage maps of *B. oleracea* based on RFLP markers have been reported (Slocum et al. 1990; Kianian and Quiros 1992; Landry et al. 1992). However, these three maps consist of loci detected by different sets of RFLP probes in distinct F_2 populations, making their integration into a common *B. oleracea* RFLP map difficult. This problem is further confounded by the highly duplicated nature of the *B. oleracea* genome (Slocum et al. 1990) and by the fact that only the polymorphic subset of the loci detected by the various RFLP probes are positioned in each of the maps. The various maps could be integrated most easily using an immortal population of true-breeding lines derived from a highly polymorphic cross. The embryogenic nature of *Brassica* microspores has made possible the rapid production of doubled-haploid lines of *B. oleracea* (Orton and Browers 1985) and *B. napus* (Lichter et al. 1988). Several populations of *B. napus* based on doubled-haploid lines have been used to generate RFLP maps (Ferriara et al. 1994; Parkin et al. 1995; Sharpe et al. 1995; Uzunova et al. 1995) but no comparable population of *B. oleracea* has been reported.

Cytological investigations have suggested that *Brassica napus* is an amphidiploid species formed from the interspecific hybridisation of *B. oleracea* (the C genome) with *B. rapa* (the A genome) (U 1935). This relationship has been confirmed by the identification of distinct linkage groups representing the A and C genomes of *B. napus* (Parkin et al. 1995). The *B. napus* population used by Parkin et al. (1995) was derived from a cross between an oilseed rape cultivar and an amphidiploid interspecific *B. rapa*×*B. oleracea* hybrid (resynthesised *B. napus*). This made it possible to distinguish the A and C genomes in the genetic map of *B. napus* by determining the diploid origin (*B. rapa* or *B. oleracea*) of the alleles from the resynthesised *B. napus* parent (Parkin et al. 1995). However, RFLP maps of *B. rapa* (Teutonico and Osborn 1994) and *B. napus* (Ferriara et al. 1994), based on the same RFLP probes but using more conventional populations, failed to identify equivalent A-genome linkage groups and no corresponding C-genome linkage groups were obvious in an earlier pair of RFLP maps of *B. oleracea* (Landry et al. 1992) and *B. napus* (Landry et al. 1991).

In the present paper we describe the production of a large population of microspore-derived doubled-haploid lines from a highly polymorphic *B. oleracea* cross. The segregation of RFLP-defined loci in this population was used to develop a high-density genetic map of *B. oleracea* and the nine linkage groups of this *B. oleracea* map were compared to the corresponding C-genome linkage groups of *B. napus* (Parkin et al. 1995).

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E. J. R Bohuon · D. J. Keith · I. A. P. Parkin · A. G. Sharpe
D. J. Lydiate (✉)
Brassica and Oilseeds Research Department, John Innes Centre, Colney Lane, Norwich NR4 7UH. UK

Materials and methods

Plant material

A single plant of a microspore-derived doubled-haploid (DH) line of *B.oleracea* ssp. italica (GDDH33: D.J. Keith, John Innes Centre, Norwich) was used to pollinate a plant from a DH line of *B.oleracea* ssp. *alboglabra* (A12DHd: D.J. Keith, John Innes Centre, Norwich) to produce a set of genetically uniform F_1 offspring. Immature buds harvested from F_1 plants were subjected to microspore-culture essentially as described by Chuong and Beversdorf (1985) except that embryos were transferred to solid culture medium when 0.5 mm in length and that haploid/doubled-haploid plants were propagated and multiplied via cuttings.

DNA extraction, Southern hybridisation and RFLP probes

DNA extraction from freeze-dried leaf material, restriction-enzyme digestion, gel electrophoresis, alkaline transfer and Southern hybridization were carried out as described in Sharpe et al (1995). A total of 167 highly informative *Brassica* RFLP probes were employed. Of these, 132 probes were also used to develop RFLP maps of *B. napus* (Parkin et al. 1995; Sharpe et al. 1995) and the remaining 35 probes were derived from the same libraries of cloned *Brassica PstI* fragments (Sharpe et al. 1995).

Genetic analyses

Genetic linkage analysis was performed using MapMaker versions 1.9 and 3.0 (Lander et al. 1987). A minimum LOD score of 5.0 was used to associate RFLP loci into linkage groups. Three-point and multi-point analyses were used to establish the most-probable locus order for each linkage group. The final order of loci was determined after extensive proof reading, by minimising the number of double crossovers flanking single loci. Recombination frequencies were converted to map distances using Kosambi's mapping function (Kosambi 1944). The significance of differences in recombination frequency, in the corresponding intervals of linkage maps based on different populations, was estimated using chi^2 tests on two-way contingency tables of parental and recombinant genotypes.

Results

Genetic linkage analysis using a population of doubled-haploid lines

Microspore-culture was carried out on material from approximately 100 genetically uniform F_1 plants and populations of haploid/doubled-haploid lines derived from distinct F_1 plants were kept separate. The four largest populations, derived from plants F_1-15, F_1-22, F_1-34 and F_1-45 and consisting of 28, 36, 47 and 58 lines, respectively, were used to produce a combined population of 169 lines. This combined population was subjected to RFLP analysis using 167 informative probes and 303 polymorphic loci were detected (an average of 1.8 polymorphic loci per probe). The frequency of probes detecting varying numbers of polymorphic loci is represented in Fig. 1. Fifty four percent of RFLP probes detected more than one polymorphic locus (in the *B. oleracea* mapping population) and a high proportion of probes detected additional monomorphic bands on the genomic Southerns of the population indicating that calculations based on polymorphic loci will probably underestimate sequence duplication. Both parental alleles were identified at 256 (84%) of the loci scored. These loci exhibited normal disomic inheritance with one or the other of the parental alleles present in each line (Fig. 2). It was only possible to score the presence or ab-

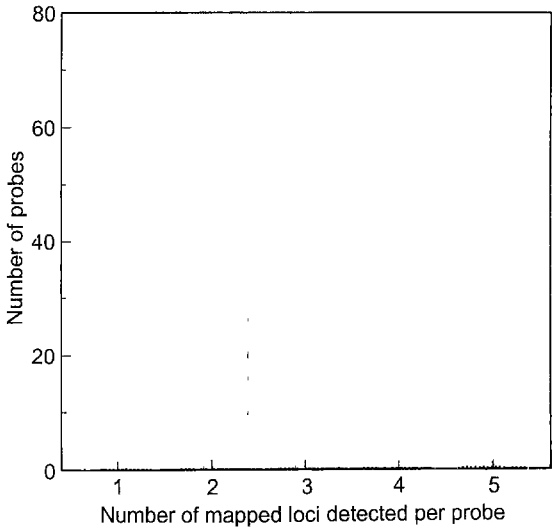

Fig. 1 The degree of duplication in the *B. oleracea* genome is illustrated by the frequency of RFLP probes detecting different numbers of polymorphic loci in the *B. oleracea* population derived from the A12DHd × GDDH33 cross

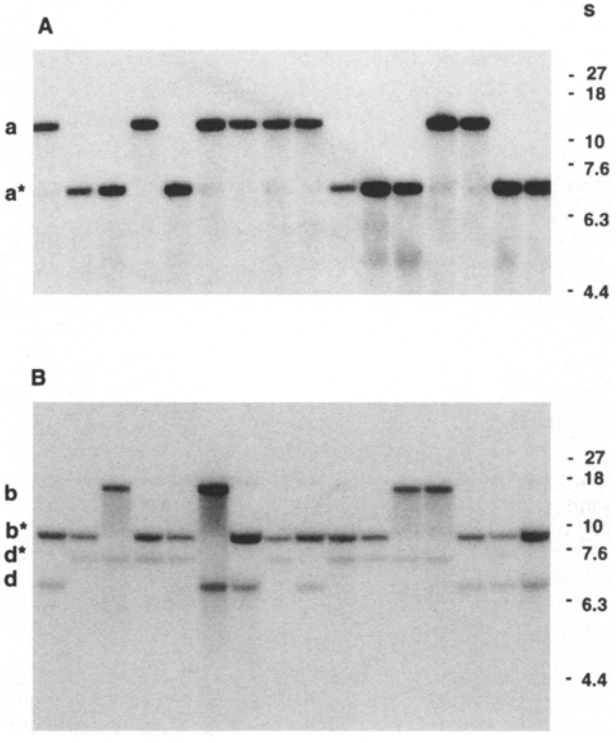

Fig. 2A, B Autoradiographs of RFLP alleles segregating in 16 DH *B. oleracea* lines from the mapping population. **A** A single polymorphic locus with alleles a and a* was detected by probe pW233. **B** Two polymorphic loci with alleles b/b* and d/d* were detected by probe pO160. *. denotes alleles from the GDDH33 parent. *Numbers* to the right of the autoradiographs represent the positions of the size standards (sizes in kilobase pairs)

sence of the allele from one parent at the remaining 47 loci (16%). There were no instances where both parental alleles of any locus were found in the same line. This indicated that none of the microspore-derived lines arose from unreduced gametes and that none of the lines carried newly duplicated chromosomal segments. Similarly, all lines contained one or the other parental allele at every locus for which both alleles could be scored, indicating that none of these loci had been eliminated or deleted. Genetic-linkage analysis produced a map containing all 303 polymorphic loci arranged in nine extensive linkage groups with a total length of 875 cM (Fig. 3). The nine linkage groups probably correspond to the nine chromosome pairs of *B. oleracea*.

Comparative analysis of the C genomes of *B. oleracea* and *B. napus*

One-hundred and thirty two of the RFLP probes used in the above analysis of *B. oleracea* had been used previously to characterise a segregating population of *B. napus* derived from a cross between an oilseed rape cultivar and a resynthesised *B. napus* line (an amphidiploid interspecific hybrid between *B. rapa* and *B. oleracea*) (Parkin et al. 1995). It was possible to distinguish the A- and C-genome linkage groups of the genetic map of *B. napus* derived from this cross because the diploid origin (*B. rapa* or *B. oleracea*) of the alleles from the resynthesised *B. napus* parent could be determined and the C-genome linkage groups were designated N11 to N19 (Parkin et al. 1995).

The common set of 132 probes detected 231 polymorphic loci in *B. oleracea* and 168 polymorphic loci in the C genome of *B. napus*. The high frequency with which RFLP probes detected multiple loci in the *Brassica* C genome (Fig. 1) made it necessary to base the initial alignment of the *B. oleracea* and *B. napus* C-genome maps on 103 pairs of corresponding loci each with at least one RFLP allele in common between the two species. The *B. oleracea* ssp. alboglabra genomes of the A12DHd parent of the *B. oleracea* cross and the resynthesised parent of the *B. napus* cross (a chromosome-doubled interspecific *B. rapa* ssp. chinensis × *B. oleracea* ssp. *alboglabra* hybrid: Parkin et al. 1995) frequently shared common alleles and the 103 loci with common alleles were distributed across all nine of the C-genome linkage groups. The inte-

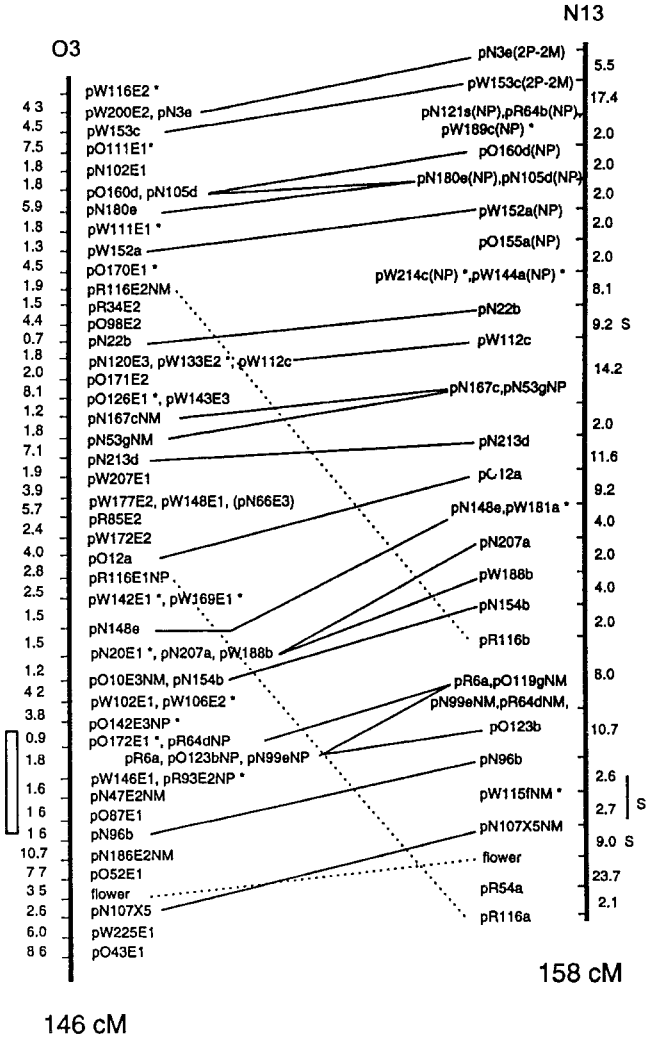

Fig. 3 Genetic linkage maps of the *Brassica* C genome in *B. oleracea* and *B. napus*. Linkage groups (*vertical lines*) are arranged in corresponding pairs and the linkage groups on the left of each pair (O1–O9) are from the *B. oleracea* map. The linkage groups on the right of each pair (N11–N19) are from *B. napus* maps: N11–N15 and N17–N19 are derived from the segregation of marker loci in the N61-9 population (Parkin et al. 1995) while N16 is from the N61-13 sister population (I.A.P.Parkin, unpublished): N16 was monosomic in the F_1 individual from which the N61-9 population was derived. RFLP-defined loci are represented by the code for the appropriate RFLP probe. The pC probes were derived from a cDNA library of *B. napus* while pN, pO, pR and pW probes were derived from libraries of genomic *Pst*I fragments derived from *B. napus* in the case of the pN and pW libraries, from *B. oleracea* in the case of the pO library and from *B. rapa* in the case of the pR library. The *flower* locus on O3 and N13 controls flower colour and the allele for white flowers is dominant to the allele for yellow flowers. On *B. napus* groups, the probe name is followed by a *lower case letter* to distinguish different loci recognised by the same probe. The same *lower case letters* are used on the *B. oleracea* groups where corresponding loci have been identified. Where loci are unique to the *B. oleracea* map the different loci recognised by the same probe are distinguished by „E" followed by a *number*. A pair of segregating alleles was scored at each locus except for loci with identifiers ending in *NP* or *NM* where only the allele from the A12DHd or GDDH33 parent, respectively, could be scored. All loci that were separated by recombination are represented with even spacing. Map distances in cM are on the outsides of each pair of linkage groups. Corresponding loci with equivalent positions in both *B. oleracea* and *B. napus* are connected by *solid lines* while the six pairs of loci that might be equivalent but which map to different locations are joined by *dotted lines*. *=loci detected in one population by probes that were not used to characterise the other population. S, SS and SSS denote intervals on the *B. napus* map where the recombination fractions differed from those in the corresponding intervals of the *B. oleracea* map to degrees expected to occur by chance with probabilities $P<0.05$, $P<0.01$ and $P<0.001$, respectively. *Open bars* indicate regions where markers were clustered in the C-genome maps of both *B. oleracea* and *B. napus*. *Shaded bars* indicate regions where markers were clustered in *B. oleracea* but not in *B. napus*

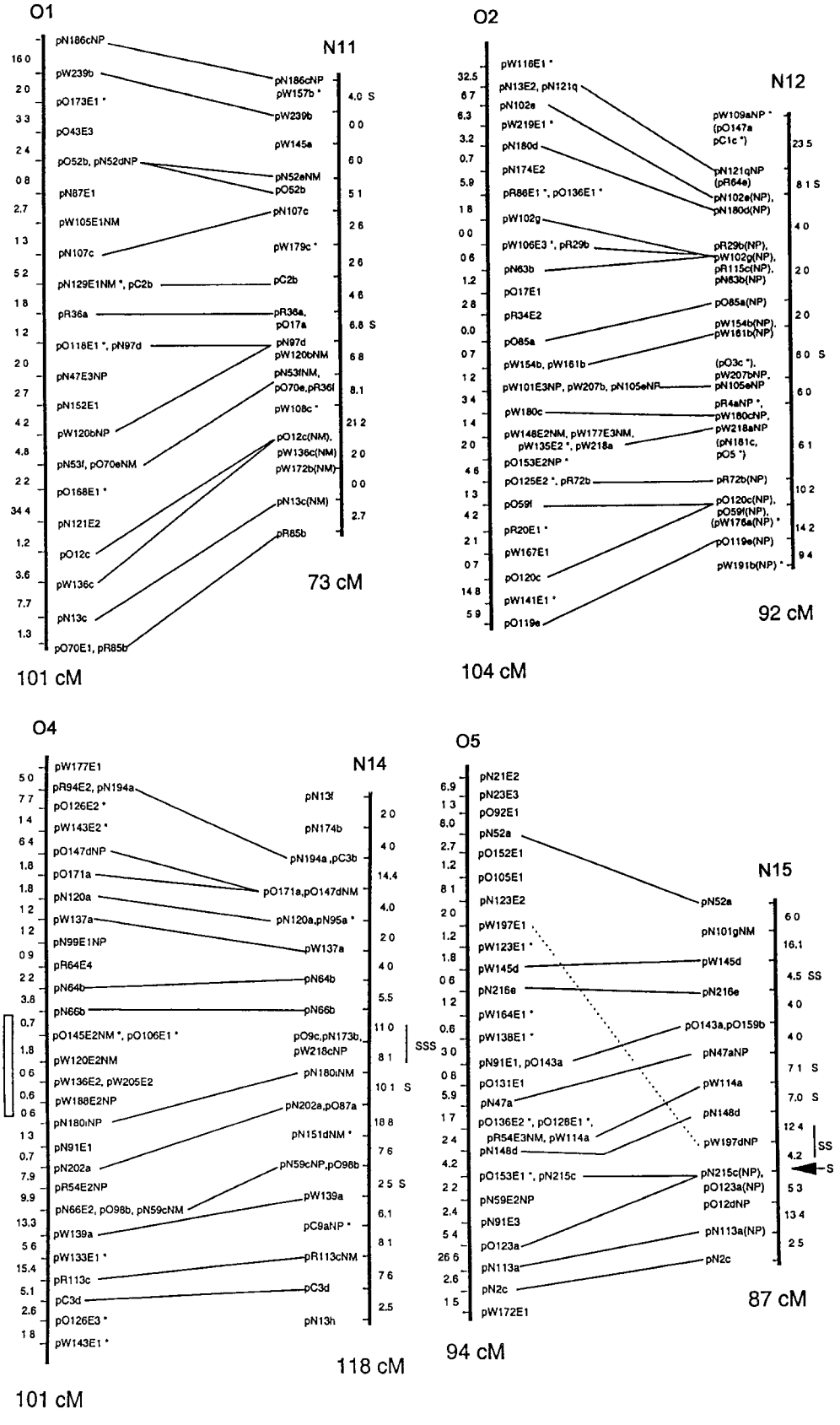

Fig. 3
Legend of figure 3 please see on page 835

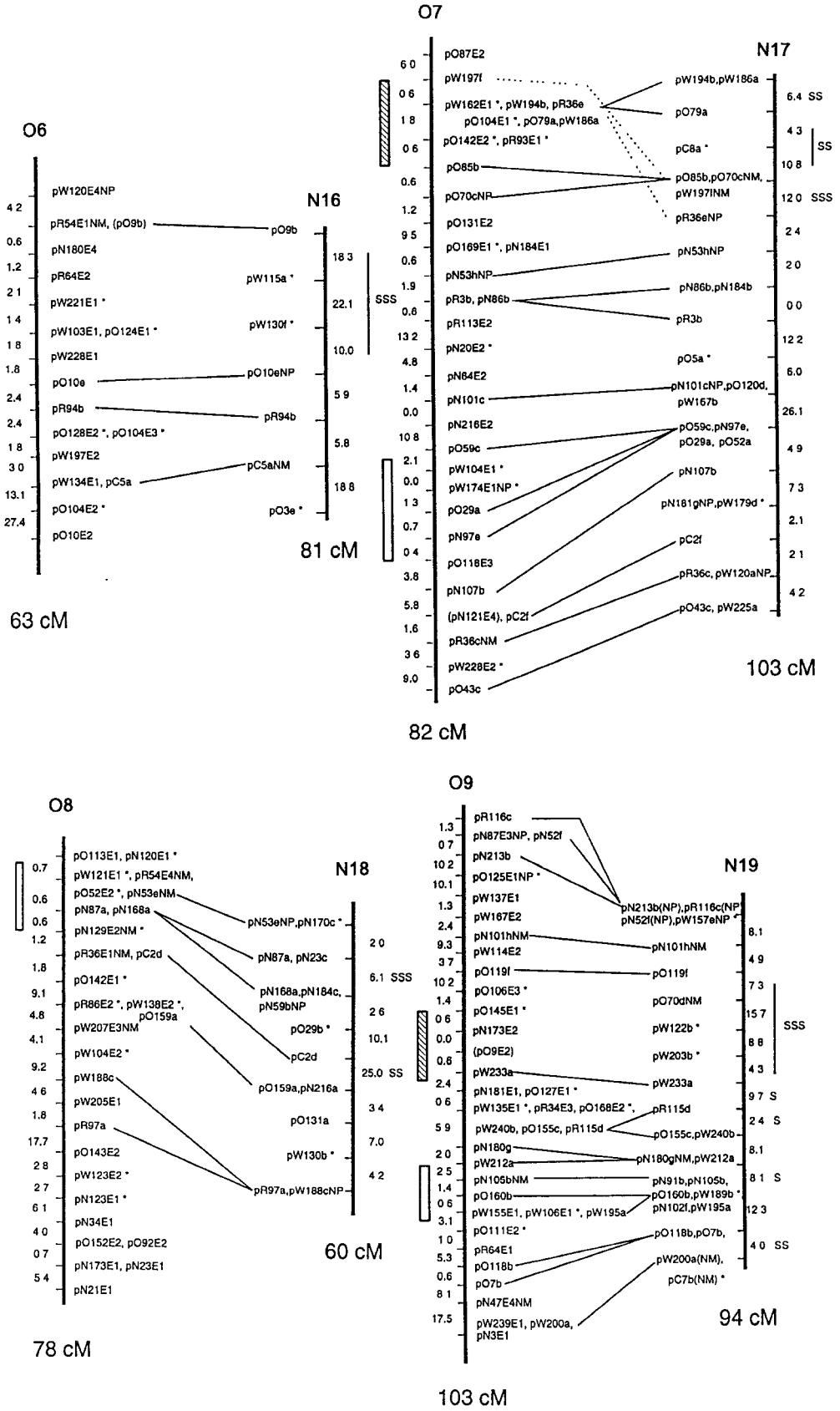
O6
pW120E4NP
pR54E1NM, (pO9b)
pN180E4
pR64E2
pW221E1 *
pW103E1, pO124E1 *
pW228E1
pO10e
pR94b
pO128E2 *, pO104E3 *
pW197E2
pW134E1, pC5a
pO104E2 *
pO10E2
63 cM
N16
pO9b
pW115a *
pW130f *
pO10eNP
pR94b
pC5aNM
pO3e *
SSS
81 cM
O7
pO87E2
pW197f
pW162E1 *, pW194b, pR36e
pO104E1 *, pO79a,pW186a
pO142E2 *, pR93E1 *
pO85b
pO70cNR
pO131E2
pO169E1 *, pN184E1
pN53hNP
pR3b, pN86b
pR113E2
pN20E2 *
pN64E2
pN101c
pN216E2
pO59c
pW104E1 *
pW174E1NP *
pO29a
pN97e
pO118E3
pN107b
(pN121E4), pC2f
pR36cNM
pW228E2 *
pO43c
82 cM
N17
pW194b,pW186a
pO79a
pC8a *
pO85b,pO70cNM,
pW197fNM
pR36eNP
pN53hNP
pN86b,pN184b
pR3b
pO5a *
pN101cNP,pO120d,
pW167b
pO59c,pN97e,
pO29a, pO52a
pN107b
pN181gNP,pW179d *
pC2f
pR36c, pW120aNP
pO43c, pW225a
SS
SS
SSS
103 cM
O8
pO113E1, pN120E1 *
pW121E1 *, pR54E4NM,
pO52E2 *, pN53eNM
pN87a, pN168a
pN129E2NM *
pR36E1NM, pC2d
pO142E1 *
pR86E2 *, pW138E2 *,
pO159a
pW207E3NM
pW104E2 *
pW188c
pW205E1
pR97a
pO143E2
pW123E2 *
pN123E1 *
pN34E1
pO152E2, pO92E2
pN173E1, pN23E1
pN21E1
78 cM
N18
pN53eNP,pN170c *
pN87a, pN23c
pN168a,pN184c,
pN59bNP
pO29b *
pC2d
pO159a,pN216a
pO131a
pW130b *
pR97a,pW188cNP
SSS
SS
60 cM
O9
pR116c
pN87E3NP, pN52f
pN213b
pO125E1NP *
pW137E1
pW167E2
pN101hNM
pW114E2
pO119f
pO106E3 *
pO145E1 *
pN173E2
(pO9E2)
pW233a
pN181E1, pO127E1 *
pW135E1 *, pR34E3, pO168E2 *,
pW240b, pO155c, pR115d
pN180g
pW212a
pN105bNM
pO160b
pW155E1, pW106E1 *, pW195a
pO111E2 *
pR64E1
pO118b
pO7b
pN47E4NM
pW239E1, pW200a,
pN3E1
103 cM
N19
pN213b(NP),pR116c(NP)
pN52f(NP),pW157eNP *
pN101hNM
pO119f
pO70dNM
pW122b *
pW203b *
pW233a
pR115d
pO155c,pW240b
pN180gNM,pW212a
pN91b,pN105b,
pO160b,pW189b *
pN102f,pW195a
pO118b,pO7b,
pW200a(NM),
pC7b(NM) *
SSS
SS
94 cM

Fig. 3

gration of the C-genome maps on the basis of the 103 pairs of equivalent loci with shared alleles identified a further 26 pairs of equivalent loci with distinct alleles. Thus the common set of 132 probes detected a total of 270 different polymorphic loci in the C genomes of *B. oleracea* and/or *B. napus* with 129 loci common to both maps (Fig. 3). The major cause of 102 (of the 270) loci being unique to the *B. oleracea* map and 39 (of the 270) loci being unique to the *B. napus* map was probably a limitation in the degree of polymorphism rather than the deletion and/or insertion of loci in either species. In almost every case where a polymorphic locus was detected by one of the 132 common probes in only *B. oleracea*, a monomorphic band (possibly representing the same locus) was observed in *B. napus*. The *B. oleracea* locus *pN99E1NP* was the one exception to this rule. Similarly, when a polymorphic locus was detected by one of the common probes in only *B. napus*, a monomorphic band was observed in *B. oleracea*.

The linear order of equivalent loci along the corresponding C-genome linkage groups of *B. oleracea* and *B. napus* exhibited a high degree of conservation, with 123 out of 129 loci collinear (Fig. 3). Based on this comparison, no gross structural alterations differentiated the C-genome chromosomes of the two species (with the possible exception of the top of O7). The high level of agreement between the two maps indicated not only the conserved nature of the genome but also the high level of accuracy with which the linear orders of loci had been determined in both maps. Of the six pairs of loci that were not collinear; *flower* (O3/N13) and *pW197dNP* (N15) might have been anomalous due to scoring errors, *pW197f* and *pR36e* might have been in a region of O7 that was rearranged in one or other of the parents of the *B. oleracea* cross and it is possible that different loci were detected by pR116 on O3 and N13.

The total length (in centiMorgans) of the regions of the C genome bordered by collinear loci in the *B. oleracea* and *B. napus* maps was fairly constant, 702 cM and 779 cM, respectively. Furthermore, over half of the difference in genetic size was due to the unusual behaviour of the chromosome represented by O6/N16. However, comparing the length of each of the 111 intervals flanked by pairs of collinear loci revealed that 24 intervals (21%) were different to an extent expected to occur by chance at a frequency of 5% and that five of these intervals (4.4%) were different to an extent expected to occur by chance in only 0.1% of intervals (Fig. 3).

Discussion

A genetic linkage map of *B. oleracea* consisting of 303 loci was produced, with all loci arranged on nine linkage groups spanning a total of 875 cM. The length of this map was similar to that of the other most complete *B. oleracea* genetic linkage map, 820 recombination units (Slocum et al. 1990), and was in reasonable agreement with the lengths of the other (less complete) linkage maps, 1112 cM (Landry et al. 1992) and 747 cM (Kianian and Quiros 1992), suggesting that an average of one crossover per chromatid is standard for *B. oleracea*. A more detailed comparison of the different genetic linkage maps of *B. oleracea* was impossible because different RFLP probes were used to characterise the different populations. The population described above, consisting of 169 immortal DH lines derived from the progeny of a highly polymorphic *B. oleracea* ssp. alboglabra×*B. oleracea* ssp. italica cross, will be an excellent reference population for the integration of loci detected by different marker systems into a common genetic map. This population will be made available to *Brassica* geneticists and it is hoped that it will be adopted not only to determine the relative positions of loci detected by different sets of RFLP probes but also to integrate AFLP loci, RAPD loci and microsatellite loci into the existing RFLP map.

The striking collinearity between the C-genome linkage groups of *B. oleracea* and those of *B. napus* reinforced the conclusion of Parkin et al. (1995) that the structures of the C-genome chromosomes are essentially identical in the two species. The highly significant compressions (Fig. 3) on *B. oleracea* linkage groups O7 (top) and O9 (middle) with respect to the equivalent regions of the corresponding *B. napus* linkage groups, N17 and N19, respectively, might represent segments of *B. oleracea* chromosomes where one parent carries an inversion. It is possible that the major clusters of marker loci common to the maps of *B. oleracea* and *B. napus*, and particularly those on linkage groups O3/N13, O4/N14, O7/N17, O8/N18 and O9/N19 (Fig. 3), surround the centromeres of the corresponding chromosomes. The nine linkage groups of the *Brassica* C genome probably correspond to nine distinct chromosome pairs, and cytological studies (based on lines of *B. napus* monosomic for one or more C-genome chromosomes) are being carried out to test this hypothesis (A. Kelly and D. Lydiate, unpublished).

The linkage groups of the *B. oleracea* map described above (Fig. 3) were numbered and orientated so as to match the corresponding linkage groups of *B. napus*: O1=N11, O2=N12, ... and O9=N19. This numbering system will make it easier to identify corresponding linkage groups in related species and thus simplify the identification of equivalent loci influencing the same trait. There would be obvious advantages to the universal adoption of this nomenclature for *B. oleracea* linkage groups. A system for numbering seven of the nine chromosomes/syntenygroups of *B. oleracea* and based on monosomic addition lines was proposed by McGrath et al. (1990). However, the structural instability of the monosomic chromosomes of such addition lines (Hu and Quiros 1991) compromises their use as long-term reference material.

The population of 169 homozygous lines, all extensively characterised with RFLP markers, is an ideal resource for analysing the genetic and environmental control of quantitative traits. The mapping of loci detected by a common set of RFLP probes (Thormann et al. 1994), using the population described above and an F_2 population derived from a cabbage×broccoli cross (Camargo et al.

1996), will allow the integration of the genetic maps derived from the two populations. This will facilitate the comparative mapping of quantitative trait loci controlling a number of traits (most notably flowering-time: Camargo and Osborn 1996) in the two populations.

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