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Analysis of RFLP mapping inaccuracy in *Brassica napus* L.

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Abstract We identified sources of mapping inaccuracy during the construction of RFLP linkage maps from one F_2 population and two F_1 microspore-derived populations from the same cross of oilseed *Brassica napus*. The genetic maps were compared using a total of 145 RFLP marker loci including 82 loci common to all three populations. In the process, we identified a series of mapping events that could lead to ambigous conclusions. Superimposed restriction fragments could be mistaken as a single dominant restriction fragment in a F_2 population and, when analyzed as such, would yield inaccurate linkage information. Residual heterozygosity in parental lines resulted in complicated allelic assignment and yielded subsequent difficulties in linkage determination. Loose and spurious linkages occurred during mapping and were identified by comparing maps derived from different populations. LOD scores and χ^2 test of independence were compared for their capacity to detect loose linkages or generate spurious ones. Extreme segregation distortions towards the same parental allele also contributed to an additional source of spurious linkage. Small but significant segregation distortions resulted in reduced estimates of the recombination fraction. The use of the same 'probe × enzyme' combinations in doubled haploid populations allowed the identification of the correct allele assignment as well as loose and spurious linkages. A translocation between two homoeologous linkage groups was observed. The consequences of such a chromosomal event as a source of error in mapping applications are discussed.

Key words *Brassica napus* · Linkage map · Mapping inaccuracy · RFLP · Segregation distortion

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Introduction

Genetic linkage maps have been constructed for several crop species. Doubled haploid (DH), backcross, single-seed descent (SSD), F_2 and F_3 populations, and recombinant inbred lines have been used for segregation analyses. A variety of morphologic and isozymic markers, restriction fragment length polymorphism (RFLP) markers, random amplified polymorphism DNA (RAPD) and other polymerase chain reaction (PCR) based markers such as amplified fragment length polymorphism (AFLP) have been used successfully for map construction (Vos et al. 1995).

The availability of detailed genetic maps has been the corner-stone for several new areas of research in plant genetics. Identification of markers linked to Mendelian and quantitative trait loci (QTL) of agronomic importance has been achieved using co-segregation and QTL analyses, and linked markers are now being used in marker-assisted selection programs (Tanksley et al. 1989; Paterson et al. 1991). Physical mapping of a trait followed by isolation of the underlying gene is now possible through map-based cloning (Martin et al. 1993a, b). For all these purposes, reliable genetic maps become essential.

Most published maps have been based on segregation data from a single population derived from a single

cross. Comparative mapping has been used to show extensive synteny between different species of the Solanaceae (Gebhardt et al. 1991; Prince et al. 1992; Tanksley et al. 1992), between maize and sorghum (Binelli et al. 1992; Pereira et al. 1993), between maize and rice (Anh and Tanksley 1993), between wild and cultivated rice (Jena et al. 1993), between mungbean and cowpea (Menancio-Hautea et al. 1993), between wheat, rye and barley (Devos et al. 1993) and between *Arabidopsis thaliana* and *Brassica oleracea* (Kowalski et al. 1994). The approach has not been applied widely to compare maps of a species produced from different mapping populations. In oilseed crops, barley, maize and rice, however, multiple maps have been constructed from different crosses and population types (Shin et al. 1990; Beavis and Grant 1991; Graner et al. 1991; Heun et al. 1991; Landry et al. 1991; Song et al. 1991; Bentolila et al. 1992; Chyi et al. 1992; Kleinhofs et al. 1993; Jena et al. 1993; Murigneux et al. 1993; Nagamura et al. 1993; Wang et al. 1994). Because these maps originated from different research groups, comparative mapping was only performed for a few populations. Composite maps, that is one map of a species drawn with segregation data from several populations, were, most of the time, not constructed. Efforts are now being made to combine several of the existing barley maps and *Brassica* linkage maps, which will lead to the production of a more detailed and accurate composite map of the species (A. Graner and C. F. Quiros, personal communications).

The construction of linkage maps is a stepwise process when multipoint linkage analysis is performed. Each step is associated with a decision-making process and has the potential to be misleading. The LOD score is a frequently used statistic to test for linkage and to assign linkage groups. A pairwise LOD score compares the likelihood of linkage versus independent segregation between two markers. In multipoint linkage analyses, a LOD score of 3 corresponds to an odd first-type error of 0.001 or 10^{-3} (Gerber and Rodolphe 1994). The choice of a critical value for LOD score should take into account the population type and size, and the nature of the genetic markers. Therefore, in a study including 100 markers, analysis of a total of 4950 pairwise comparisons ($100 \times (100 - 1)/2$) is performed. Approximately five first-type errors ($4950 \times 1/1000$), linking markers that are not linked, would occur with a critical value of 3 for LOD score. Tanksley et al. (1992) used a LOD threshold of 5 in the making of high-density maps of potato and tomato for which more than 1000 markers were included. The LOD score, when used as a threshold for deciding linkage or not, depends directly on the number of markers analyzed and should be adjusted accordingly to minimize first-type error and to efficiently detect true linkages. More stringent LOD scores are often used to build framework maps and less stringent ones to assign less informative or loosely linked markers to linkage groups.

An additional source of mapping errors is related to segregation distortion, a phenomenon that has been reported in several species (Foisset and Delourme 1996). The effect of segregation distortion at a single locus on mapping accuracy has been studied in maize DH, F_2 and SSD populations, where it was found to affect the detection of linkage using LOD scores (Bentolila et al. 1992; Murigneux et al. 1993). Yet, most maps have so far been constructed using LOD score statistics to assemble markers into linkage groups without special regard for markers showing distorted segregation.

Comparative mapping can be a powerful tool to expose mapping errors. In maize, loose and spurious linkages were identified by comparing linkage (Bentolila et al. 1992; Murigneux et al. 1993). The effect of single-factor segregation distortion on the determination of linkage using χ^2-test of independence and LOD score has also been estimated (Murigneux et al. 1993).

In the study reported here, we compared three mapping populations of the same cross to see how they display the *B. napus* genetic map. One map was constructed from a F_2 population and two others from microspore-derived populations. We evaluated several parameters, including the effects of segregation distortions and the significance of statistical tests as potential mapping errors. Wrong allelic assignments due to residual heterozygosity or superimposition of alleles are also suggested as a source of inaccuracy for the resulting linkage map. Potential mapping errors were identified from actual mapping data as opposed to simulated data. Strategies to detect the errors and solutions to correct them are proposed.

Materials and methods

Plant materials

A population of 90 F_2 plants was obtained by selfing a F_1 from a cross between cv 'Topas' and cv 'Westar' (Landry et al. 1991). The microspore-derived populations were provided by Dr. L. S. Kott and Dr. W. D. Beversdorf of the Department of Crop Science, University of Guelph, Ontario, Canada. These populations, named M3 and M5, were obtained by culturing in vitro the microspores of 2 other F_1 plants obtained from the same cross. Microspore culture techniques were as described previously (Kott et al. 1987). Spontaneous diploid plants were selfed, and resulting dihaploid lines that were found not to be 100% homozygous according to their RFLP patterns were not included in the segregation analyses. The F_1 microspore-derived population M3 consisted of 8 haploid plants and 36 dihaploid lines whereas F_1-microspore-derived population M5 was composed of 3 haploid plants and 40 dihaploid lines. Plants were grown in the greenhouse as previously described (Landry et al. 1991). Leaves were periodically harvested and immediately stored at $-80^{\circ}C$ until DNA extraction.

Source of probes

Eighty cDNA clones designated 1ND1 to 4NG11, 6 seedling-specific cDNA clones (pCa15, pCot1, pCot39, pCot44, pGs43, pAx92)

provided by Dr. J. J. Harada and a cruciferin cDNA clone (pC1) provided by Dr. M. Crouch, most of which were polymorphic in the F_2 population, were used as probes on all three segregating populations (Landry et al. 1991). Plasmids were prepared using the Magic minipreps DNA purification system (Promega, USA), and cDNA inserts were purified with Geneclean™ kit (Bio 101, USA or Sephaglas band prep kit, Pharmacia, Canada).

Southern blot analysis

Genomic DNA was isolated, purified and digested as described by Landry et al. (1991). Only 5 µg of DNA, however, was loaded per lane. Restriction enzymes *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII were used, and several duplicates of each genomic restriction digest were made to perform up to ten hybridizations simultaneously. After digestion and separation by electrophoresis on agarose gel, DNA was transferred onto Hybond™-N^+ membranes (Amersham, UK) by capillarity (Southern 1975). The alkaline transfer procedure (Reed and Mann 1985) with 0.4 *M* sodium hydroxide was also done as recommended by the manufacturer of the membrane (Amersham, UK).

DNA inserts were labelled with [^{32}P] with T7 Quick Prime™ kit (Pharmacia, Canada). Hybridization conditions were as described by Landry et al. (1991) with the exception that an additional 10% (w/v) dextran sulfate was added to the hybridization solution.

Identification of polymorphic clones and nomenclature of loci

Polymorphic cDNA probes were first identified by comparing DNA banding patterns between the parental lines. *B. napus* cv 'Topas' and cv 'Westar' are not 100% homozygous (Cloutier et al. 1995). Only 82 loci, however, were common to all three populations. The others were either common to two of the three populations or unique to one population because of the residual heterozygosity within parental cultivars (see below).

When the restriction fragment profiles were consistent and clearly readable in at least two populations, association of a specific RFLP to a locus was possible among populations because the same 'probe × enzyme' combinations were used for all populations. Superimposed loci and translocations were identified by comparing RFLPs of the three populations (see results). Duplicated loci detected by a single clone were designated by the same probe name followed by a different lowercase letter as described in Landry et al. (1991).

Segregation ratios and linkage analyses

Goodness-of-fit within expected Mendelian segregation ratios were calculated for each locus within each population using Linkage-1 software (Suiter et al. 1983). Chi-square tests of independence were also derived using Lingake-1, and pairwise LOD scores were obtained from MapMaker Version 2.0 software (Lincoln et al. 1990).

Maps were constructed on a Macintosh LC III using MapMaker Version 2.0 software (Lincoln et al. 1990). Independent mapping was performed for each of the three populations. Linkage groups were assigned with criteria for a recombination frequency of 0.30 and LOD 4.0. Markers forming groups consistent in all three populations were used to built framework maps by multipoint mapping analysis using a threshold of LOD 3.0. Remaining markers with a LOD of 2.0 or more in at least two populations were assigned sequentially by trying them in each interval. Loose and spurious linkages were identified by comparing the three resulting maps.

Results

Effects of superimposed restriction fragments on mapping accuracy

The segregation patterns obtained by the hybridization of clone 3NH3 displayed three polymorphic DNA restriction fragments (RFI, RFII, RFIII) and one monomorphic restriction fragment (Fig. 1). RFI and RFIII were inherited from cv 'Topas' and RFII was from cv 'Westar'. With the F_2 segregation data only, there was no evidence to suggest that RFII was not allelic to RFI or RFIII. However, if RFII was considered as an allele to one of them, an extremely skewed segregation ratio in favor of 'Westar' alleles would have resulted. The lack of solid evidence to clearly designate the RFII as an allele of either RFI or RFIII has resulted in scoring the three fragments as three dominant loci. As such,

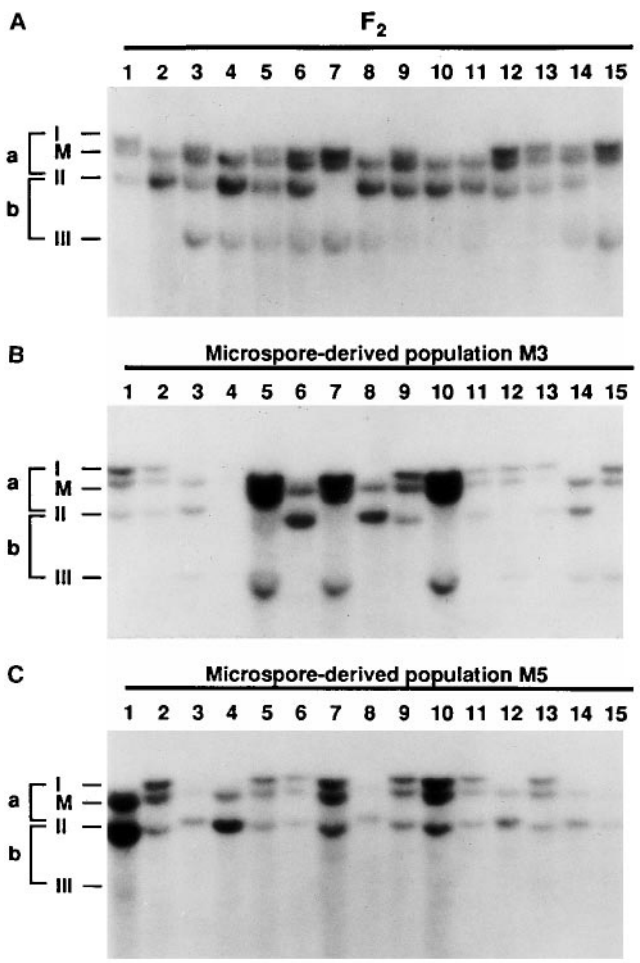

Fig. 1A–C Restriction patterns obtained by hybridization of cDNA clone 3NH3 to 15 individuals (*lanes 1–15*) of **A** population F_2, **B** microspore-derived population M3 and **C** microspore-derived population M5. Monomorphic (*M*) and polymorphic restriction fragments (RF*I*, RF*II*, RF*III*) as well as locus assignment (*a*, *b*) are identified

RFI and RFIII fitted expected Mendelian segregation ratios, but RFII showed an extreme segregation bias towards 'Westar' alleles and fitted a 15:1 ratio in population F_2. Using the F_2 data, pairwise LOD scores of the 'locus' defined by RFII was 1.63 with the locus defined by RFI and 1.91 with the locus defined by RFIII.

Hybridization of the same clone (3NH3) to two microscope-derived populations revealed the superimposition of two identical alleles of two loci inherited from 'Westar'. They were unresolved with RFII (Fig. 1B,C), since both alleles of RFII were associated to RFI and RFIII that came from 'Topas'. The locus defined by RFII segregated according to a 3:1 ratio in microspore-derived population M3 and was monomorphic in microspore-derived population M5.

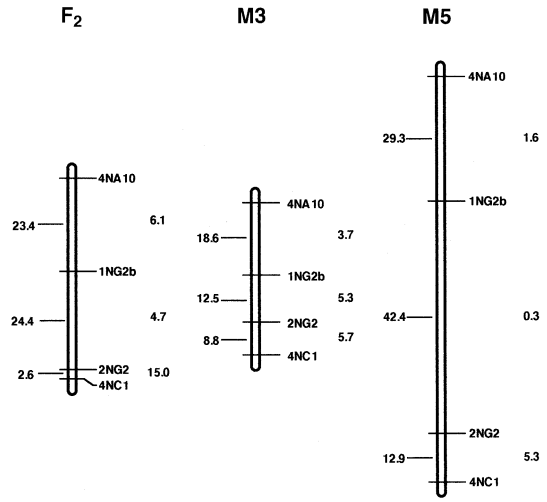

Fig. 2 Conserved order but different estimates of distances between markers common in all three segregating populations. Linkage group from population F_2 (*left*), microspore-derived populations M3 (*center*) and M5 (*right*). Distances in recombination units are displayed to the *left* of the linkage groups and pairwise LOD scores are to the *right*

Uncertain allelic phase assignment as a consequence of residual heterozygosity in parental lines

The parental lines we used for mapping contained a variable level of residual heterozygosity estimated to be between 20% and 23% for cv 'Topas' and between 11% and 13% for cv 'Westar' (Cloutier et al. in preparation). We identified residual parental heterozygosity at some loci as a source of mapping errors. Assignment of the allelic phase of a given DNA restriction fragment was not always possible and could vary among our three segregating populations because they were derived from three independent F_1 individuals. We assigned both phases for each allele at each locus and verified which phase yielded a marker consistently linked to the same linkage group in all populations. We used this approach to identify the allelic assignment of marker 4NE8a in the F_2 population (data not shown). This approach, however, did not work for markers showing extreme segregation bias. For example, the ambigous allelic assignment of marker pCa15, which showed an extremely biased segregation in the F_2 population (χ^2) = 84.9), could not be resolved with certainty because pCa15 mapped at a different location but within the same linkage group with both allelic assignments.

Loose and spurious linkages

Maps obtained from our three segregating populations and containing marker loci 4NA10, 1NG2b, and 4NC1 are illustrated in Fig. 2. Linkage was estimated for all pairs of consecutive markers for all populations using LOD scores (> 3.0) and χ^2 test of independence ($P > 0.001$). In population F_2 and M3, both statistics were significant for all pairs of loci. In population M5, pairwise LOD scores were not significant for pairs 4NA10-1NG2b and 1NG2b-2NG2 with values of 1.6 and 0.3, respectively, but the χ^2 test of independence was not significant only for pair 1NG2b-2NG2 ($\chi^2 = 1.43$, $P = 0.23$). The χ^2 test of independence was, however, significant for linkage of 4NA10 and 1NG2b ($\chi^2 = 6.93$, $P = 0.008$). A LOD score with a threshold of 3.0 and χ^2 test of independence associated with a 0.001 probability of type-I error were not equivalent in detecting loose linkages. Comparative mapping was useful to identify both loose linkages that remained undetected. Similarly, spurious linkages were identified by comparing linkage groups formed by the three populations.

Influence of segregation distortion on the recombination fraction estimates and the formation of spurious linkages

In the three populations studied, the percentage of markers showing biased segregation ratio was 29% in the F_2 population, 23% in the microspore-derived population M3 and 31% in the microspore-derived population M5 (Cloutier et al. 1995). While some markers showed slight segregation bias (e.g. pCot1a in population M5; $\chi^2 = 5.2$, $P = 0.02$), others showed considerable distortion (e.g. 2ND9 in population F_2: $\chi^2 = 95.7$, $P < 10^{-6}$). Two examples drawn from the data of our three *B. napus* mapping populations illustrate the effect of the magnitude of segregation bias on linkage analyses.

In the first example, goodness-of-fit to expected Mendelian segregation ratios of 7 RFLP markers are shown for the three populations of *B. napus* (Table 1). In the F_2 population, all these markers exhibited extreme segregation bias towards 'Topas' alleles and were

Table 1 Comparison of one-locus and two-locus statistics including χ^2 test of independence and LOD scores. Statistics for a group of (a) 7 RFLP markers showing extreme segregation distortion in the F_2 population but not in the microspore-derived populations, and (b) 8 linked RFLP markers showing segregation distortion of a lower magnitude in microspore-derived population M5 only

Marker	Goodness-of-fit to expected Mendelian segregation ratio			Two-locus statistics					
				F_2		M3		M5	
	F_2	M3	M5	χ^2	LOD[a]	χ^2	LOD	χ^2	LOD
a)									
2NE3	32.5**	0.2	(−)	162.6**	40.4	36.0*	10.8	(−)	(−)
3NE1	40.6**	0.4	(−)	28.8**	8.5	2.1	0.47	(−)	(−)
3NB3	48.9**	0.1	1.4	43.3**	16	(−)	(−)	(−)	(−)
4NA1c	39.2**	(−)	(−)	32.2**	11.9	0.1	0	7.2**	0.9
4NA1d	36.0**	1.9	1.5	25.4**	9.8	(−)	(−)	(−)	(−)
4ND4b	31.0**	(−)	(−)	34.0**	11.9	2	0.5	0.01	0.1
3NC3	37.3**	0.8	5.2*						
b)									
pCot1	4.5	0.3	5.2*	26.9**	5.9	13.4**	3.2	18.1**	5
3NC7a	0.3	0	7.0**	45 **	12.1	22.2**	6	35.0**	10.5
1NF7b	0.7	0.9	9.0**	62.3**	12.7	29.0**	8.7	34.0**	10.2
1ND1a	0	0	6.4	46.6**	9	7.2**	1.6	20.4**	5.3
2NE1	0.1	0	8.8**	64.8**	10.7	18.0**	4.4	18.1**	5.6
2NC8b	2.3	0	10.3**	21.6**	3.8	16.4**	3.9	17.6**	5.3
2NB10c	0.4	0.4	7.8**	1.7	0.3	10.8**	2.6	16.3**	4.3
2NB10b	2	0	0.2						

*$0.05 < P < 0.01$, **$P < 0.01$
[a]LOD3 ($P = 10^{-3}$)

linked with significant pairwise LOD scores between 8.5 and 40.4. In population M3, 5 of these 7 markers were polymorphic and segregated according to their 1:1 expected segregation ratio. Only 3 of these markers, however, were polymorphic in population M5 and segregated normally, except marker 3NC3 that showed segregation bias towards 'Westar' alleles. In population M3, only markers 2NE3 and 3NE1 were linked. In populations M3 and M5, we had no evidence to confirm linkage of the other markers. With significant pairwise LOD scores ranging from, 3.2 to 8, marker 3NB3, however, was linked to a different linkage group as in population F_2 (Fig. 3). In population F_2, the extremely distorted segregation of marker 3NB3 resulted in a spurious linkage with other markers also showing extremely distorted segregation towards the same 'Topas' allele.

For the second example, goodness-of-fit to expected Mendelian segregation ratios for markers pCot1a, 3NC7a, 1NF7a, 1ND1a, 2NE1, 2NC8b, 2NB10c and 2NB10b are shown in Table 1 for all three populations. While the 8 markers segregated according to expected Mendelian ratios in populations F_2 and M3, 7 of them showed segregation distortion in population M5. The magnitude of the segregation distortion of the 7 markers was small but significant ($5.2 < \chi^2 < 10.3$). These 8 markers, however, maintained their linkage assignment in all three populations (Fig. 4). Low-magnitude segregation distortion of the 7 markers did not affect the detection of proper linkage in population M5. A reduced estimate of the recombination fraction, however, was observed. The linkage group had a total length of 110 cM in the F_2 population, 94 cM in population M3 and only 50 cM in population M5.

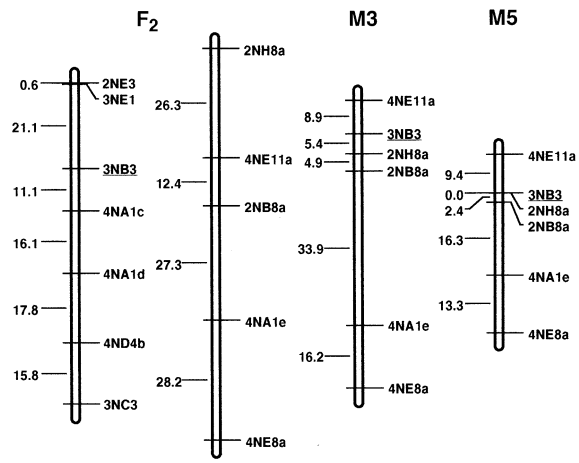

Fig. 3 Formation of spurious linkages as a result of strong segregation distortions. The two linkage groups on the *left* were drawn from a F_2 population data and show positioning of marker 3NB3 with markers showing strong segregation bias. Marker 3NB3 which showed a Mendelian segregation in the microspore-derived populations was assigned to a different linkage group in population M3 (*center*) and M5 (*right*). Recombination units are displayed to the *left* of the linkage groups

Mapping errors resulting from chromosomal rearrangements

Hybridization of cDNA clones 2NB9, 2NG12, pCot44 and 4ND3, each produced two polymorphic restriction fragments in the F_2 population and microspore-derived population M3. In the F_2 population, the two polymorphic fragments produced by each one of these

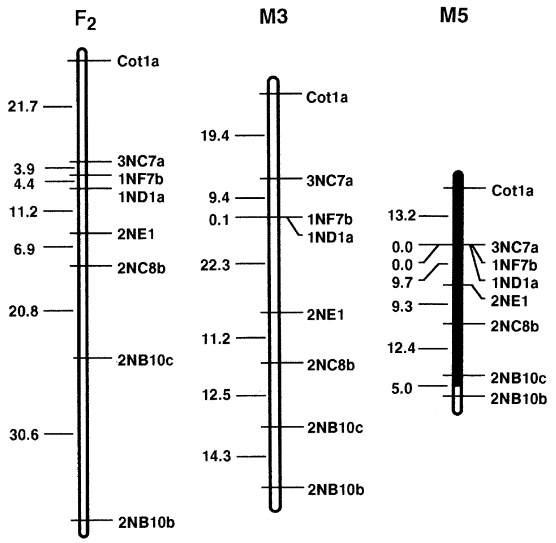

Fig. 4 Effect of low-magnitude segregation distortion on the recombination fraction estimates. Map configuration of a linkage group composed of 8 RFLP markers derived from a F_2 population (*left*), microspore-derived populations M3 (*center*) and M5 (*right*). Recombination units are displayed to the *left* of the linkage groups. A *solid black bar* within the linkage group illustration indicates the location of markers showing segregation distortion in favor of 'Topas' alleles

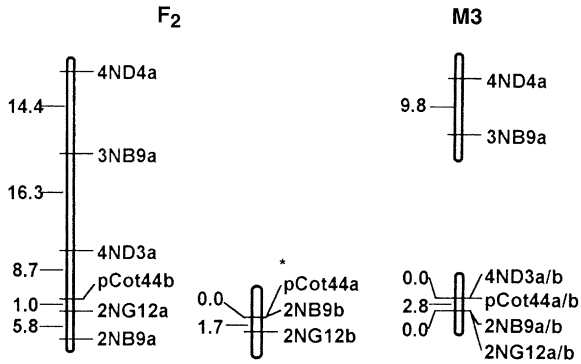

Fig. 5 Translocation event illustrated by comparison of linkage groups formed by 6 RFLP markers for the F_2 population (*left*) and microspore-derived population M3 (*right*) *4ND3b could not be mapped in population F_2 because of a poor hybridization signal

4 cDNA clones were not allelic because both restriction fragments were absent in some F_2 plants. In consequence, they were scored as dominant/null markers and mapped to two distinct linkage groups in this population (Fig. 5). The same two polymorphic restriction fragments, however, were allelic in microspore-derived population M3. Such RFLP patterns in two distinct F_1-derived populations are indicative of a translocation. The strand break was located between marker 3NB9a and 4ND3a.

Discussion

Assignment of alleles

In a F_2 population, allelic association of DNA fragments is determined by the presence of one, the other or both allelic restriction fragments. If two restriction fragments are allelic, both fragments must not be absent in any given F_2 plant. Complicated patterns, however, may be observed when multiple-copy clones are hybridized. Polyploid species such as *B. napus* often produce multi-banded restriction patterns (Landry et al. 1991). Such patterns may be difficult to interpret, especially with F_2 segregation patterns. Genetic ratios are simpler with DH plants since only two classes are present (Henderson and Pauls 1992; Siebel and Pauls 1989). In DH segregating populations, two DNA restriction fragments are allelic only if a plant exhibits only one or the alternate allelic form and never displays both or none of the DNA fragments.

Superimposed alleles that could not be identified in the F_2 population were revealed when the same 'probe x enzyme' combination was hybridized to two F_1 microspore-derived populations obtained from the same cross. Absence of heterozygous individuals in F_1 microspore-derived populations resulted in simplified restriction patterns and facilitated the identification of superimposed allelic fragments that could not be clearly assigned in the F_2 population.

While pairwise LOD scores of less than 2.0 are normally not considered sufficient evidence for linkage between 2 loci, LOD scores of more than 1.5 between dominant markers detected by a same clone could be an indication of superimposition of alleles rather than independent loci. RFLP segregation ratios can also serve as an indicator of allele superimposition. In a F_2 population, dominant RFLPs normally fit a 3:1 Mendelian segregation ratio. A significant segregation ratio of 15:1 is another indication of superimposition of alleles. In a DH population, any RFLP showing a significant 3:1 segregation ratio potentially represents also two alleles. When undetected, wrong allelic assignment could, therefore, yield erroneous linkage information.

When two heterozygous parents are crossed four possible F_1 genotype can be produced for each heterozygous locus. Among the possible populations derived from these F_1 plants, two would display monomorphic hybridization patterns and two would yield polymorphic and identical patterns if the parental lines carried the same alleles. Phase assignment of the polymorphic and identical fragments would, however, be impossible because the polymorphic fragments can be from either parent.

Assignment of the two possible phases of the alleles and comparison of linkage groups can be used as a strategy to determine the correct phase of alleles

when they segregate according to Mendelian ratios. DNA of the 2 plants that served as parents of the F_1 hybrid should also be added to blots of the segregating population during hybridization whenever they are available. Identification of the parental origin of the restriction fragment would then be possible when only 1 of the 2 parental plants used in the cross was heterozygous. In most studies, polymorphism is assessed by comparing hybridization patterns of the two parental lines. Two heterozygous parents would not be polymorphic and the segregation would likely not be tested. Wrong phase assignment due to residual heterozygosity may again result in inaccuracy in the vicinity of the marker locus wrongly scored.

In species where F_1-derived haploid and dihaploid plants can be produced, the problem of parental residual heterozygosity can be bypassed. In *B. napus*, successful microspore culture has been achieved for a number of genotypes and could be used to produce DH plants of the two parental cultivars (Chuong et al. 1987). The parents and the F_1 from which the segregating population is derived would then be of known genotypes, and allelic phase assignment would be unequivocal. A substantial proportion of the spontaneous diploid plants are, however, derived from unreduced gametes and, therefore, are not 100% homozygous, as we have seen here and in other species (Rivard et al. 1989). To ensure complete homozygosity, colchicine doubling of haploid plants would be preferred to spontaneous diploid plants since we have found no partially heterozygous genotypes in other populations of colchicine-doubled dihaploid populations of *B. napus*.

Linkage determination and recombination estimates

Loose and spurious linkages are likely to occur when mapping large number of markers since statistical tests for linkage are associated with type-I and type-II errors. Comparison of the consistency of linkage groups among several populations is a powerful tool to identify loose and spurious linkages. In a detailed study comparing LOD scores and χ^2, Gerber and Rodolphe (1994) found that the two tests were equivalent for extreme values (i.e. strong linkage or complete independence between loci). However, the LOD score corresponded to a more severe test and was also more conservative for the recombination fraction. In maize, partitioning of linkage groups using the LOD score gave results similar but not identical to those of the χ^2 test of independence with markers showing Mendelian segregation (Bentolila et al. 1992; Murigneux et al. 1993).

Ultimately, linkage maps will be validated by physical mapping approaches (Tanksley et al. 1992). Chromosomal addition lines are ideal for use in assigning the location of a marker to a specific chromosome. They are, however, available only for a limited number of species and do not give the linear arrangement of markers within synteny groups (Ellis and Cleary 1988; McGrath et al. 1990; Heun et al. 1991). In *B. napus*, the minute size of the chromosomes complicates their cytological characterization. Nevertheless, a karyotype of the species has been constructed (Olin-Fatih and Heneen 1992). Successful in situ hybridizations of high-copy number ribosomal DNA probes have also been performed in a number of *Brassica* species which promise to prove linkage of markers to chromosomes (Maluszynska and Heslop-Harrison 1993). While the sensitivity of detection and the complicated cytogenetics of *Brassica* species still remain a major impediment to the application of such techniques in physical mapping at present, the latter achievements are encouraging.

The percentage of loci showing significant segregation distortion vary greatly depending on the species, population type and specific cross. As many as 73% of the markers showed significant segregation distortion in a DH population of maize (Bentolila et al. 1992). In *Brassica* species, 24% (Chyi et al. 1992) and 3% (Song et al. 1991) of the markers showed skewed segregation in *B. rapa*, 12% was reported for *B. oleracea* (Landry et al. 1992) and 26% for *B. napus* (Landry et al. 1991).

Most published linkage maps have been generated using MAPMAKER software, which uses LOD scores to assign linkage groups (Lincoln et al. 1990). The assumption of Mendelian segregation at each locus, however, has often been overlooked (Murigneux et al. 1993). Linkage assignment has been determined for whole sets of markers even if they showed significant segregation distortion. The examples shown in this paper clearly illustrate the increased probability of spurious and undetected linkages with markers showing extreme segregation distortion. The use of a more stringent linkage test has been recommended for markers showing strong segregation distortion, and our data support this view (Manly 1994). The maximum recombination fraction should also be lowered significantly with small DH populations. Detection of linkage between markers with extreme segregation distortion remains, however, limited to detecting linkage among closely linked markers.

Clusters of markers with skewed segregation ratio have often been reported within linkage groups constructed in several species (e.g. Graner et al. 1991; Landry et al. 1991; Kleinhofs et al. 1993). As it is known there are increased chances of type-I error with these markers when assigned to linkage groups based on their LOD scores, prudence should be the rule when constructing linkage groups that include several markers with strongly biased segregation. Comparative mapping has been a useful tool to identify sprurious linkages here and helped to confirm true linkage between markers with skewed segregation.

Reduced recombination within chromosomal regions including markers with distorted segregation was

observed in the present study. The recombination fraction is a biased estimate when it is based on markers with skewed segregation (Bailey 1949). A systematic approach to estimate the recombination fraction between markers that accounts for differential viability (i.e. when not all genotypes are represented proportionally) has been proposed. Bailey's product formula estimates of the recombination fraction are not biased even if markers with distorted segregation are included and should be used when 2 or more consecutive markers show distorted segregation.

Chromosomal rearrangements

Brassica napus is an amphidiploid species containing the 20 chromosomes of *B. rapa* and the 18 chromosomes of *B. oleracea*. The 19 pairs of homologous chromosomes, each having a range of partial or complete homoeologues, represent its chromosomal constitution (U 1935). *B. napus* cultivars still possess sufficient affinity to allow homoeologous pairing (Newell et al. 1984). In *B. napus* pollen mother cells, multivalents were found, suggesting structural similarities between homoeologous chromosomes from the two progenitors genomes (Attia and Robbelen, 1986). Translocation between homoeologous chromosomes is not surprising even within *B. napus* cultivars.

Homoeologous regions are more susceptible to chromosomal rearrangements. Therefore, linkage maps of these regions may be less reliable, and the cross-applicability of markers in such regions for large-scale marker-assisted selection may be limited. Tightly linked and flanking markers would be advisable for application to various unmapped breeding populations.

Previous evidence indicates that *B. napus* lines originated from at least four hybridization events (Song and Osborn 1992). This information by itself leads us to forsee that a unique map of the species may not be representative. The chromosomal rearrangement detected between populations derived from the same cross further emphasized the potential artifact of a unique *B. napus* map. Some chromosomal segments may be more stable, and composite maps may be useful for marker-assisted selection and map-based cloning of these targeted regions. Other chromosomal regions may be more susceptible to recombination, which would make composite mapping obsolete. Detailed mapping in several crosses is required in *B. napus* and its progenitors to identify the regions more susceptible to rearrangement.

Conclusion

Reliable genetic maps are critical to map-based cloning and marker-assisted selection, and no map at all may be better than a false map. We have estimated several parameters that can affect the reliability of linkage maps. Warnings are issued concerning the interpretation of linkage maps depending on how they were obtained. We have advised that some precautions should be taken to identify or avoid certain mapping artifacts. We have exposed difficulties in mapping the genome of *B. napus*; these are related to its polyploidy, the residual heterozygosity of the parental cultivars, the F_2 mapping population and the origin of the species.

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