Theor Appl Genet (1998) 96: 361–366 

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# RFLP mapping of the centromere of chromosome 4 in maize using isochromosomes for 4S

Received: 19 May 1997 / Accepted: 6 October 1997

**Abstract** The centromere of maize chromosome 4 was previously localized to a 26-cM interval using molecular markers and B-A translocations. The objective of the present study was to refine the placement of the centromere using secondary trisomics. Two independently isolated secondary trisomics (having an isochromosome plus two normal homologs) for 4S were recovered. RFLP analysis of populations segregating for them placed the centromere of chromosome 4 between *bnl15.45* and *bnl7.20*, two RFLP loci that are 5.4-cM apart on the UMC map and 11.5-cM apart on the BNL map.

**Key words** Centromere · Telocentric · Secondary trisomic · Maize · RFLP

## Introduction

The centromere positions on the maize classical linkage map have been identified by traditional methods using cytogenetic variants and morphological markers and are imprecisely identified. On the current maize RFLP maps, developed by the University of Missouri (UMC) (Davis et al. 1996) and Brookhaven National Laboratory (BNL) (Matz et al. 1995), centromeres have not been accurately mapped, but approximate centromeric locations have been extrapolated from classical genetic data and the clustering of markers. The closest approximation to date has come from studies of molecular markers and B-A translocations by Weber and Helentjaris (1989). This study localized centromeres to chromosomal regions which in some cases were fairly precise. For example, two RFLP loci, *npi85* and *npi105*, which mapped to the same position on chromosome 10, were found to flank the centromere. However, for most of the chromosomes, the region that includes the centromere was tens of cM long, and in some cases over 50 cM in length. For chromosome 4, the region identified in the Weber and Helentjaris (1989) study utilizing B-A translocations was 26-cM long.

Telotrisomics (having two normal homologs plus a telocentric for one arm) and secondary trisomics (having two normal homologs plus an isochromosome for one arm) have been used to map centromeric positions in maize (Rhoades 1933, 1936, 1938, 1940), polyploid wheat (Sears 1952 a, b; Morris and Sears 1967), cotton (Endrizzi and Kohel 1966), oats (McGinnis et al. 1963), tomato (Khush and Rick 1968; Frary et al. 1996), and rice (Singh et al. 1996). Rhoades (1933, 1936, 1938, 1940) recovered a telocentric for the short arm of chromosome 5 (5S) in maize from a trisomic-5 plant. Because this "fragment chromosome" was in addition to the normal complement (2n + 5S, a telotrisomic), the plant was not genetically deficient. Rhoades utilized this telocentric to localize the centromere to a 1-cM region on chromosome 5. An interesting outcome of these experiments was the identification of exceptional individuals that were secondary trisomics (containing an isochromosome with two identical arms) for 5S (Rhoades 1933, 1940). An isochromosome arose with a low but consistent frequency whenever the telocentric chromosome was present (Rhoades 1940). The origin of this chromosome was hypothesized to be a transverse misdivision of the centromere during a pollen-grain mitosis leaving the two identical arms attached.

Communicated by G. E. Hart

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Telocentric chromosomes have originated spontaneously by transverse misdivision of the centromere from non-telocentric chromosomes when a univalent chromosome passes through meiosis (Darlington 1939, 1940; Sears 1952 b).

Doyle (1988) analyzed large numbers of selfed progeny of maize primary trisomics and identified presumptive telotrisomics on the basis of deviant genetic ratios. Two independently isolated presumptive telotrisomics for 4S obtained in Doyle's study were utilized in the current work. Both stocks contained an isochromosome for 4S in addition to two normal representatives of chromosome 4. In the current mapping study, the presence or absence of alleles of mapped RFLP loci was correlated with the presence or absence of the secondary telotrisomic for 4S so that the markers could be assigned to either the short arm or the long arm. The centromere was mapped to the region flanked by the two closest markers located in opposite arms of the chromosome.

The purpose of this study was to map the centromeric position of chromosome 4 more precisely on current RFLP maps (Davis et al. 1996; Matz et al. 1995), and to combine cytological, genetic, and RFLP data to provide a higher level of correlation among the three maps.

## Materials and methods

### Stocks

Two independently isolated presumptive telotrisomics for the short arm of chromosome 4 were recovered by Doyle (1988). Cytological analysis carried out in the current study established that both were secondary trisomics, and hereafter they will be described as iso-4Sc and iso-4Sd. For both secondary trisomics, the two normal representatives of chromosome 4 carried the recessive allele of the locus sugary, *su* (*sugary*, with wrinkled endosperm), and the 4S isochromosome carried the dominant allele, *Su* (starchy, with non-wrinkled endosperm). The dominant *Su* marker can be used to select for the presence of the isochromosome in experimental crosses. An unrelated *su*/*su* inbred stock (that was also *la*/*la*, *lazy*, with prostrate growth, and which also maps to the short arm 11-cM distal to *su*) was obtained from the Maize Genetics Cooperation Stock Center, University of Illinois, Urbana.

### Generation of telotrisomics with substituted chromosomes

The secondary trisomics were crossed as female parents with the unrelated *su la* inbred stock. Two types of kernels were produced by this cross (not considering recombination between the *Su* locus on the isochromosome and the centromere). The first type is sugary and has two normal number 4 chromosomes with *su* alleles. The second type is starchy and has two normal number 4 chromosomes with *su* alleles plus the isochromosome with the *Su* allele. Plants grown from the starchy kernels (presumptive secondary trisomics) were backcrossed as female parents by the same unrelated *su la* inbred. These crosses are diagrammed in Fig. 1. Crosses were made with a *su* inbred that was unrelated to the original secondary trisomic stock to increase the probability of finding RFLP polymorphisms. Plants with the extra isochromosome were identified by an extra band on an autoradiograph. The dominant *Su* kernels were selected as the presumptive secondary trisomics, and the *su* kernels were selected as presumptive diploids. Both of these kernel types were planted and RFLP analysis was conducted on these individuals.

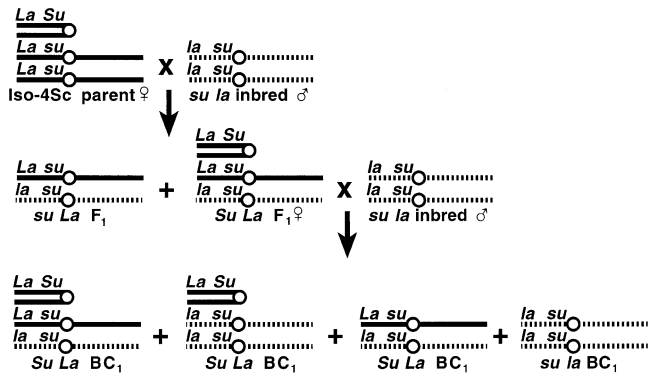


**Fig. 1** Genetic crosses made to produce secondary trisomic and diploid sibling $BC_1$ progeny in which one or both of the normal homologs are substituted

### Cytological characterization of iso-4Sc and iso-4Sd

Cytological analysis of male inflorescences undergoing meiosis in iso-4Sc and iso-4Sd plants was carried out to verify the nature of the presumptive extra arm. A portion of the immature tassel was fixed in a 3:1 mixture (v:v) of 95% ethanol: propionic acid (Sharma and Sharma 1965) at room temperature for 1 day and then maintained at $-20^{\circ}$C. Diakinesis and pachytene stages of prophase-I were stained with a propio-carmine solution. The chromosomes were observed at diakinesis of prophase-I for the presence of an extra chromosome (or chromosome fragment) in addition to the normal complement. Once a fragment was observed, pachytene preparations were made to determine if this arm contained a terminal centromere.

### Genetic data

The transmission frequencies for iso-4Sc or iso-4Sd were followed by using the dominant marker, *Su*. When a secondary trisomic having *Su* on the isochromosome and *su* alleles on the normal homologs is selfed, about one-third of the resultant kernels are expected to be plump and the remainder wrinkled. However, when a secondary trisomic is crossed as the male parent, a much smaller proportion of the kernels are expected to be plump. This is because the extra isochromosome is only infrequently transmitted through the male because unbalanced gametes compete poorly with haploid gametes.

Iso-4Sc and iso-4Sd plants were testcrossed as male and female parents, and the ratio of plump to wrinkled kernels was determined.

### RFLP analysis

Leaf samples were harvested, lyophilized, and the DNA isolated by the CTAB (mixed alkyltrimethyl-ammonium bromide) method (Saghai-Maroof, et al. 1984). Following restriction endonuclease (*Bam*HI, *Eco*RI, *Eco*RV and *Hin*dIII) digestion, the DNA fragments were separated by agarose-gel electrophoresis and blotted onto

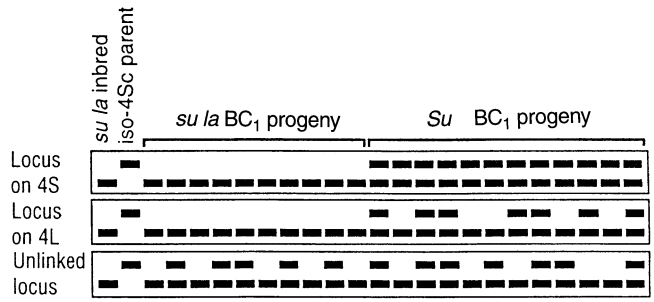


**Fig. 2** Expected results for polymorphic RFLP loci that are located in the isochromosome (on 4S), not in the isochromosome (4L), or are unlinked

a $N^+$ nylon membrane (Amersham) according to Southern (1975). Radiolabeled ($^{32}P$) maize clones that had been previously used to map RFLP loci were provided by the University of Missouri, Columbia, Mo., and Brookhaven National Lab, Upton, NY. The clones were hybridized with DNA from the presumptive telocentric individuals overnight in a 65°C Bellco rotary hybridization oven. The membranes were washed under stringent conditions (0.1 X SCP, 0.1% SDS) and exposed to Kodak X-Omat film for autoradiography.

## Results and discussion

### Cytological analysis

Microsporocyte samples from the presumptive telotrisomics 4Sc and 4Sd recovered by Doyle (1988) were analyzed at different stages of meiosis. Diakinesis cells of both 4Sc and 4Sd contained nine bivalents plus a heteromorphic trivalent (Fig. 2a) or ten bivalents plus a univalent. If 4Sc and 4Sd were telotrisomics for the short arm of chromosome 4, then one of the three members of the heteromorphic trivalent would be the length of the short arm of chromosome 4 (slightly longer than one-third of the length of a normal homolog). In each case, the length of the extra member was considerably longer than this.

At pachytene for both 4Sc and 4Sd, the extra chromosome was observed to possess two arms with similar chromomere patterns that were paired with each other along their entire lengths. A centromere could be observed at the end of the paired arms in certain cells (Fig. 2 b). In some of the cells, the centromere of the extra chromosome was fused with the centromere of another chromosome (Fig. 2 c). These observations clearly show that the additional chromosome was an isochromosome, not a telocentric chromosome. In addition, the fragment chromosome was sometimes observed in the form of a closed ring at diplotene (Fig. 2d) providing additional evidence that the extra member was indeed an isochromosome. In these cells, the two homologous arms paired and underwent recombination, and a chiasma between them held their ends together to form a closed ring.

### Genetic data

Secondary trisomic 4Sc and 4Sd plants were testcrossed as male and female parents. The results of these crosses are presented in Tables 1 and 2. The percent of *Su* kernels was significantly higher when iso-4Sc and iso-4Sd plants were testcrossed as female parents (33.06% and 36.23% respectively) than when

**Table 1** The ratio of starchy (*Su*) kernels to sugary (*su*) kernels when iso-4Sc was testcrossed either as a female or male parent

| Iso-4Sc testcrossed as female | | | Iso-4Sc testcrossed as male | | |
|---|---|---|---|---|---|
| Pedigree | *Su*:*su* | % *Su* | Pedigree | *Su*:*su* | % *Su* |
| H92-5 × 3-1 | 50:118 | 42.37 | H93-267 × 251-4 | 55:389 | 12.39 |
| H92-5 × 1-4 | 69:119 | 57.98 | H93-267 × 256-8 | 34:400 | 7.83 |
| H92-6 × 3-1 | 11:36 | 30.55 | H93-270 × 251-4 | 51:452 | 10.14 |
| H92-6 × 3-1 | 5:41 | 12.19 | H93-270 × 253-2 | 33:275 | 10.71 |
| H92-5 × 3-4 | 10:45 | 22.22 | H93-271 × 252-3 | 48:306 | 13.56 |
| Percent *Su* kernels | | 33.06 | | | 10.93 |

**Table 2** The ratio of starchy (*Su*) kernels to sugary (*su*) kernels when iso-4Sd was testcrossed either as a female or male parent

| Iso-4Sd testcrossed as female parent | | | Iso-4Sd testcrossed as male parent | | |
|---|---|---|---|---|---|
| Pedigree | *Su*:*su* | % *Su* | Pedigree | *Su*:*su* | % *Su* |
| H92-4 × 1-4 | 67:140 | 47.85 | 93-426 × 425-11 | 6:40 | 13.04 |
| H92-4 × 1-4 | 28:48 | 58.33 | 93-430 × 429-6 | 5:25 | 16.67 |
| H92-7 × 3-1 | 27:77 | 35.06 | H93-261 × 260-1 | 39:305 | 11.34 |
| H92-4 × 1-4 | 17:68 | 25.00 | H93-266 × 263-3 | 17:275 | 5.82 |
| H92-7 × 1-4 | 13:87 | 14.94 | H93-267 × 264-2 | 46:470 | 8.91 |
| Percent *Su* kernels | | 36.23 | | | 11.16 |

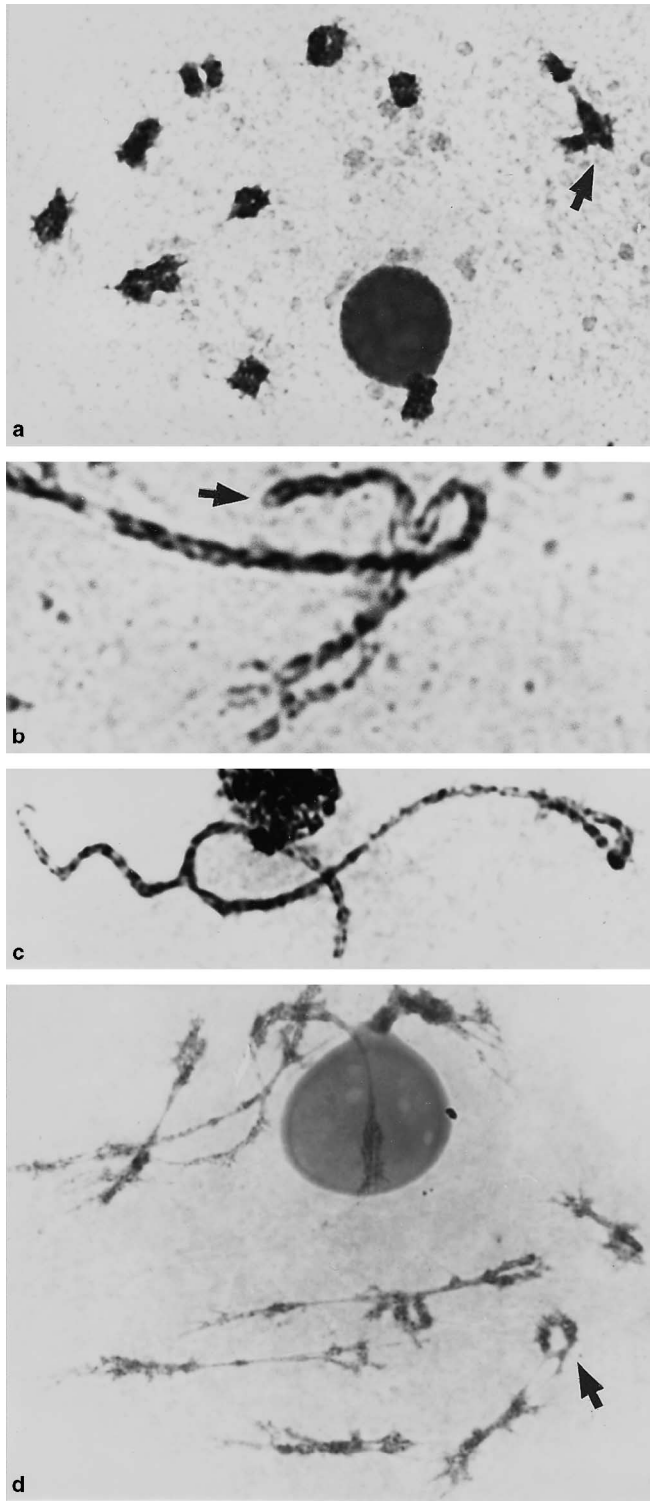


**Fig. 3a–d** Meiosis in iso-4Sc plants. **a** Diakinesis cell with nine bivalents and one heteromorphic trivalent (*arrow*). **b** The isochromosome at pachytene clearly showing the centromere (*arrow*). **c** Centromeric fusion between the isochromosome and a normal homolog (chromosome 4) at pachytene. **d** Diplotene with a ring-shaped univalent (*arrow*)

testcrossed as male parents (10.39% and 11.16% respectively). The genetic ratios obtained from these crosses with both presumptive secondary trisomics are similar to the genetic ratios obtained with other isochromosomes and telocentrics (Rhoades 1936, 1940; Khush and Rick 1968; G. Doyle, unpublished for telo-6La), and distinctively different from the ratios expected from trisomics or from diploid heterozygotes. The genetic data produced by a study of this type would be very similar for a telotrisomic or a secondary telotrisomic. The transmission frequencies of the "telocentric" through the male in this study were nearly ten-fold higher than observed by Rhoades (1940) for telo-5S, or Doyle (unpublished) for telo-6La. This apparent inconsistency may be because secondary telotrisomics generally are more stable than telocentrics (Rhoades 1940; Khush and Rick 1968) and therefore would be transmitted more often.

## RFLP analysis

The analysis included: (1) a population of 14 *Su* $BC_1$ progeny (secondary telotrisomics) and ten *su la* $BC_1$ progeny (controls) for iso-4Sc, and (2) a population of 13 *Su* individuals (secondary telotrisomics) and ten *su la* individuals (controls) for iso-4Sd. The original telotrisomics were homozygous for the dominant allele, *La*. The lazy (*la*/*la*) plants grown from sugary kernels were selected as controls because they would have both chromosome 4s from the *su la* parent (not considering recombination between the marker loci and the RFLP locus being analyzed).

DNAs of the iso-4Sc and iso-4Sd parental lines and the *su la* inbred were each digested with four different restriction enzymes (*Bam*HI, *Eco*RI, *Eco*RV, and *Hin*dIII). They were then each screened with cloned sequences for 39 RFLP loci located within 80 cM of the centromeric region. The 1995 Brookhaven National Laboratory (BNL) and the 1996 University of Missouri (UMC) RFLP maps were used to identify appropriate RFLP loci. Eight of these revealed polymorphisms between iso-4Sc and iso-4Sd, and the *su la* inbred. These were then used to analyze blots containing DNAs of *Su* and *su la* $BC_1$ progeny for iso-4Sc and iso-4Sd. In addition to the mapping information, it was learned that the banding patterns resulting from the parental screens were not identical. The fact that some of the probes identified fragments of different molecular weights in the original iso-4Sc and iso-4Sd parents provides additional support that iso-4Sc and iso-4Sd originated independently.

## Mapping rationale

Figure 3 presents the three mutually exclusive banding patterns that will be produced by RFLP loci on: (1) the

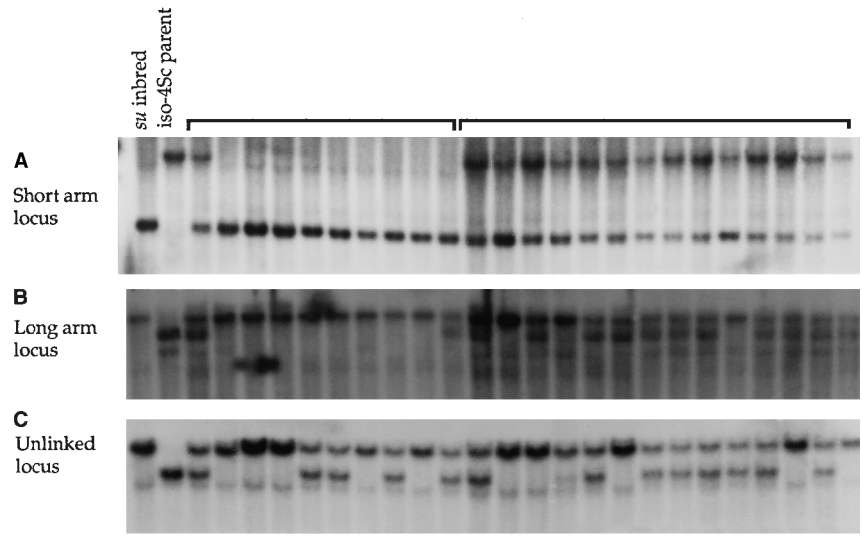


**Fig. 4** RFLP banding patterns produced by RFLP loci that are in 4S, 4L, or are unlinked. **a** *npi386*, a 4S locus. **b** *bnl7.20*, a 4L locus. **c** *bnl12.06*, an unlinked segregating locus

short arm of chromosome 4 (covered by the isochromosome), (2) the long arm of chromosome 4, and (3) another chromosome (not considering recombinants). If the RFLP locus is on the short arm of 4, all *Su* $BC_1$ progeny will display two bands (the one that was present in the original telotrisomic and the one from the *su la* inbred) and all *su la* individuals will only display the band from the *su la* inbred. Because all *su* $BC_1$ individuals had been selected for *la*, nearly all would be expected to only display the band from the *su la* inbred parent. If the locus was on the long arm, approximately half of the *Su* individuals will display two bands and the *su la* individuals will display only the allele from the *su la* inbred. If the marker is unlinked, then half of both the *Su* and *su la* $BC_1$ progeny will display two bands with every individual displaying the *su la* allele. Based on this rationale, every polymorphic marker can be unequivocally assigned to the short arm of 4, the long arm of chromosome 4, or another chromosome. The centromere would then map to the region between the closest two markers that are assigned to different arms.

## RFLP analysis of iso-4Sc $BC_1$ progeny

RFLP probes bnl5.46, npi386, umc42, npi263, and bnl15.45 each produced a pattern of bands that indicated that they identify loci located in the short arm of chromosome 4. All *Su* individuals displayed the fragment that was present in the *su la* inbred line and also the fragment that was present in the original iso-4Sc parent. The *su la* $BC_1$ individuals only displayed the fragment from the *su la* inbred. An example of a RFLP locus that produced a banding pattern of this type is shown in Fig. 4a.

RFLP probes npi270, and bnl7.20 each produced a pattern of bands that indicated that they identify loci on the long arm of chromosome 4. All of the $BC_1$ progeny displayed the fragment that was present in the *su la* inbred, and approximately half of the *Su* $BC_1$ progeny displayed the fragment that was present in the original iso-4Sc parent. An example of a RFLP locus that produced a banding pattern of this type is shown in Fig. 4 b.

Two RFLP probes (umc14 and bnl12.06) previously used to map loci in chromosome 4 produced banding patterns that indicated that the locus identified did not mapped to this chromosome. All of the $BC_1$ progeny (both *Su* and *su la*) displayed the fragment that was present in the *su la* inbred, and approximately half of the $BC_1$ progeny (both *Su* and *su la*) also displayed the fragment that was present in the original iso-4Sc (Fig. 4 c). It is important to note that both of these probes identified one monomorphic and one polymorphic RFLP locus. It is possible that the monomorphic locus in both cases is the locus in chromosome 4 and that the polymorphic locus is in another chromosome. We now understand that much of the maize genome is present in duplicate (Helentjaris et al. 1988; Ahn and Tanksley 1993), and that a large portion of maize probes identify more than one RFLP locus. This occurred with these two RFLP loci.

## RFLP analysis of iso-4Sd $BC_1$ progeny

*Su* and *su la* $BC_1$ progeny for iso-4Sd were analyzed with the same RFLP probes. The same segregation patterns were found for each of the RFLP loci as was found for iso-4Sc $BC_1$ progeny. The fact that these two isochromosomes for 4S which arose independently gave identical results provides an increased level of confidence in the mapping of this centromere.

## Location of the centromere

From this study, the RFLP loci closest to the centromere on the short and long arms, respectively, are

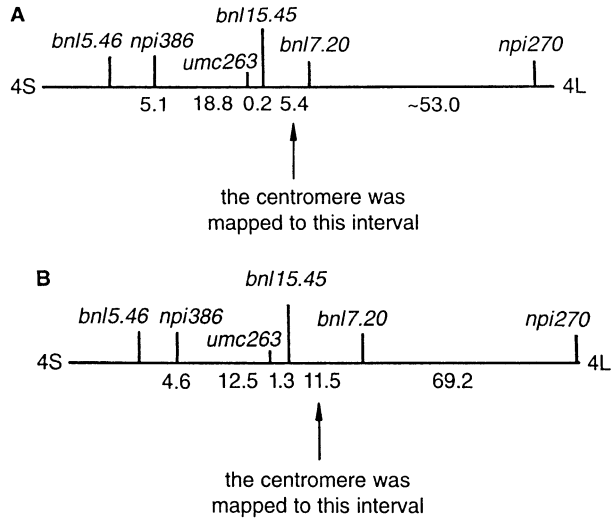


**Fig. 5** Location of the centromere on chromosome 4 of the BNL 1995 (**A**) and UMC 1996 (**B**) RFLP maps

*bnl*15.45 and *bnl*7.20. The distance between these markers is 11.5 cM on the 1996 BNL RFLP map (Fig. 5). Although *bnl*7.20 is not positioned on the 1996 UMC RFLP map, M. McMullen (University of Missouri, personal communication) indicated that recent mapping data indicates that it is 5.4 cM from *bnl*15.45; thus, the centromere is in a 5.4-cM interval between these markers on this map.

**Acknowledgments** This work was supported by a grant to D.W. from the USDA, #92-37300-7592, and a cooperative agreement from the USDA-ARS, #58-3622-1-133, to Ed Coe. We thank Novartis Seeds Inc. for providing summer nursery facilities in which part of this material was grown and M. McMullen and T. Musket at the University of Missouri for mapping RFLP loci on the UMC RFLP map.

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