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Excision and transposition of two *Ds* transposons from the *bronze mutable* 4 Derivative 6856 allele of *Zea mays* L.

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Summary. The regulatory mutation *bronze mutable* 4 Derivative 6856 (*bz-m4* D6856) contains a complex 6.7 kb *Dissociation* (*Ds*) element tagged with a duplication of low copy *bz* 3′ flanking sequences (Klein et al. 1988). This creates a unique opportunity to study the transposition of a single member of the repetitive family of *Ds* elements. Eighteen full purple revertants (*Bz′* alleles) of *bz-m4* were characterized enzymatically and by genomic mapping. For 17 of the *Bz′* alleles, reversion to a wild-type phenotype was caused by excision of the 6.7 kb *Ds* transposon. Nine of these *Bz′* alleles retained the transposon somewhere in their genome. In this study we show that like *Ac* (Schwartz 1989; Dooner and Belachew 1989), the 6.7 kb *Ds* element can transpose within a short physical distance, both proximal and distal to its original position. Additional *bz* sequences have been mapped immediately distal to the mutant locus in *bz-m4* D6856; genetic evidence suggests these are flanked by two additional *Ds* elements. The remaining *Bz′* revertant, *Bz′*:107, arose from excision of a more complex 13 kb *Ds* element.

Key words: *Zea mays* – *bronze* – Transposable elements – *Ds*

Introduction

Ds elements can exist in 30 or more copies per genome (Nevers et al. 1986), making it difficult to monitor individual *Ds* transpositions. A 6.7 kb *Ds* transposon in *bz-m4* D6856 is "tagged" with low copy number *bz* 3′ flanking sequences (Klein et al. 1988). This tag creates a unique opportunity to follow a specific *Ds* element at the molecular level. In addition it allows us to compare the transposition behavior of the non-autonomous element *Ds* to that of the autonomous element *Ac*.

It has been possible to follow excision and transposition of the autonomous element *Ac* throughout the maize genome due to dosage effects of *Ac* (Greenblatt and Brink 1962; Greenblatt 1984) and the low copy number of intact *Ac* elements (Fedoroff et al. 1984). *Ac* elements which had excised from the *Pericarp* locus were found to re-insert frequently on the same chromosome (Van Schaik and Brink 1959; Greenblatt and Brink 1962; Greenblatt 1968, 1984). Greenblatt (1984) interpreted the distribution of map positions of the transposed *Ac*s to mean that *Ac*s transpose during chromosome replication: an *Ac* excises from a newly replicated chromatid segment and asymmetrically transposes into an unreplicated segment, thereby defining the size and relative position of the replicon. Later, Chen et al. (1987), interpreted molecular data to support this model.

Recently, however, Schwartz (1989) and Dooner and Belachew (1989) analyzed *Ac* transpositions, from *waxy* and *bz* respectively, and observed that *Ac* can re-insert very close by, both proximal and distal to its original insertion site. These data imply *Ac* can transpose to both replicated and unreplicated chromosome sites. The repetitive nature of *Ds* elements, however, precludes direct genetic and molecular analysis of *Ds* transposition (Kermicle et al. 1989).

The *Bronze* gene in *Zea mays* codes for the enzyme UDP-glucose:3-O-flavonol glucosyltransferase (UGFT). This enzyme catalyzes one of the final steps in anthocyanin biosynthesis (Larson and Coe 1977; Dooner and Nelson 1977a). In wild-type (*Bz*) kernels the aleurone layer is purple in color. In the *bronze* mutations lacking UFGT activity, the anthocyanidins (aglycone form) break down as the tissue ages. This results in the characteristic bronze pigmentation in the aleurone layer of kernels, and in other plant tissues.

The first *bz-m4* allele arose concomitantly with a stable deletion of the *Sh* locus (McClintock 1956, 1965; Klein et al. 1988). McClintock has isolated various derivatives from the original *bz-m4* (B. McClintock, personal communication). The mutant phenotype of *bz-m4* D6856 is caused by the insertion of a 6.7 kb *Ds* in the 5′ untranslated leader region of the locus (Klein et al. 1988). Mapping a genomic clone of *bz-m4* D6856 showed this "transposon" had two *Ds* elements and intervening DNA corresponding to a duplication of *bz* 3′ flanking sequences. We use the term transposon because of the similarity of this complex *Ds* element to type II bacterial transposons (Kleckner 1981). The 6.7 kb transposon in the *bz-m4* D6856 allele affects both the level and the tissue-specific localization of *Bz* expression (Dooner and Nelson 1977b; Dooner 1981; Kuhn and Klein 1987; L. Paje-Manalo and A. Klein, unpublished results).

In this report we show that there are a total of four *Ds* elements at the *bz* locus in the *bz-m4* D6856 allele. Two of these *Ds* elements (1 and 2 in Fig. 1) delineate the 6.7 kb complex *Ds* transposon described by Klein et al. (1988). The two remaining *Ds* elements (3 and 4 in Fig. 1) are downstream of the 6.7 kb *Ds* transposon. These *Ds* elements

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flank a 4.8 kb piece of DNA which is a tandem duplication of *Bz* coding sequences and 3′ flanking sequences. A 13 kb *Ds* transposon is delineated by the first *Ds* and the third *Ds*.

Seventeen independent germinal revertants (*Bz′* alleles) from *bz-m4* D6856 were isolated. One other full purple revertant was obtained from Dr. Hugo Dooner (1981). Only two types of *Ds* excisions lead to the restoration of the wild-type phenotype. Seventeen revertants resulted from the excision of the 6.7 kb *Ds* transposon from the untranslated leader region of the *Bz* locus. In nine of these *Bz′* lines, the transposon was still present within the genome: the 6.7 kb *Ds* transposon was found re-inserted both proximal and distal within several kilobase pairs of its original position in *bz-m4* D6856. The remaining revertant probably arose via excision and loss of a 13 kb *Ds* transposon. Excision of the 13 kb *Ds* transposon restored *Bz* to a single-copy locus by removing one copy of *Bz* coding sequences.

Materials and methods

Enzymes and reagents. Restriction endonucleases were purchased from Bethesda Research Laboratory (Bethesda, Md), New England Biolabs (Beverly, Mass) and Promega Biotech (Madison, Wis). [^{32}P]dATP and dTTP were purchased from New England Nuclear (Boston, Mass). Random Priming kits were purchased from Boehringer Mannheim (Indianapolis, Ind). Zeta-Probe and Gene-Tran nylon membranes were purchased from Bio-Rad (Richmond, Calif) and Plasco (Woburn, Mass) respectively. HPLC standards, quercetin and isoquercitrin, were purchased from Extrasynthese (Genay, France).

Selection of Bz′ (m*4 D6856*) *revertants.* A line homozygous and stable for the *bz-m4* D6856 allele was crossed with *bz-R Ac^wx-m9* pollen (*bz-R* is the reference, null allele; *Ac^wx-m9* has an autonomous *Ac* element at the *waxy* locus, 25 map units proximal to *bronze* on chromosome 9S). The F_1 plants were self-pollinated and individual, full purple kernels were selected from the F_2 seed. These are designated *Bz′* revertants, after the convention used by Dooner (1981); this indicates the revertant allele is not necessarily identical at the molecular level to the progenitor allele (*BzMcC*). Revertant lines were self-pollinated for two or three additional generations to eliminate the *Ac* bearing chromosome and to isolate homozygous *Bz′* lines.

Genomic DNA extraction. Immature tassels (3 to 5 g fresh weight) were collected from 7 week old plants homozygous for *bz-m4* D6856, *BzMcC* (wild-type) or one of the revertant alleles. Genomic DNA was isolated according to the method of Shure et al. (1983).

Southern blot analysis. Approximately 10 µg of maize genomic DNA was digested for 2 h with 50 units of restriction endonuclease, under conditions specified by the suppliers. After digestion, samples were concentrated against *n*-butanol, and salts were removed by dialysis (P. Anderson, personal communication): the samples were pipetted onto 0.025 µm, 8 mm diam., VS filters (Millipore, Milford, Mass) which were floated on 1 l of TE pH 8.0 (TE = 10 mM TRIS-Cl buffer pH 8.0, 1 mM EDTA) for 2 h. Alternatively, the digested DNAs were precipitated in isopropanol at room temperature and resuspended in 1 × TBE (1 × TBE = 50 mM TRIS-borate buffer pH 8.1, 1 mM EDTA; M. Murray, personal communication). The dialysis or precipitation of the DNAs was undertaken to minimize the effect of salts, from the restriction digests, on the electrophoretic mobility of the restriction fragments. DNAs were fractionated on 0.6% agarose gels (40 V, 12–16 h). Radiolabeled lambda DNA fragments (*Hin*dIII or *Bst*EII) were included as molecular weight markers. DNAs were transferred to nylon membranes according to the manufacturers' instructions for capillary transfer. DNAs were fixed to the membranes by UV irradiation using a modification of the method of Khandjian (1986). Membranes with transferred DNA were rinsed in 10 × SSC (10 × SSC = 1.5 M NaCl, 150 mM sodium citrate pH 7.0). While still damp, the membranes were placed on a glass plate with the DNA side of the membrane up and covered with a sheet of Saran Wrap. The membrane sandwich was places on a Fotodyne (New Berlin, Wis) UV light box (800 µW/cm^2 at 0.5 cm, 254 nm) for 5 min. After UV treatment, the membranes were blotted dry and baked at 80° C, under vacuum, for 1 h. UV treated membranes were stripped and reprobed up to six times. Genomic fragment sizes varied as much as 15% between gels, therefore control samples, usually *BzMcC* or *bz-m4* DNAs, were included on all gels.

Subcloned fragments (Fig. 1) of pMBzR1, the genomic clone of the *BzMcC* allele (Furtek 1986), were labeled with ^{32}P by the random priming method (Feinberg and Vogelstein 1983). The specific activity of probes ranged from 0.5–1 × 10^9 dpm/µg DNA. Hybridizations were carried out at 42° C in 50% formamide, with 10% dextran sulfate added, according to the instructions for DNA-DNA hybridization on Zeta-Probe membranes (Reed 1986). Membranes were washed at 70° C for 15 min successively in 2 × SSC, 0.1% sodium dodecyl sulfate (SDS), 0.5 × SSC, 0.1% SDS and 0.1 × SSC, 0.1% SDS. The final wash was for 30 min in 0.1 × SSC, 1% SDS. Autoradiography was carried out at −70° C with Dupont Cronex Quanta III intensifying screens for 1 to 4 days. Fragment sizes listed in Table 1 are those measured for individual Southern blots. Sizes given in parentheses are those measured for cloned DNAs (Furtek 1986; Klein et al. 1988) where sizing is expected to be more accurate. In the text and in restriction maps, we refer to fragment sizes as predicted from the cloned DNAs.

Enzyme extractions. All revertant alleles and *BzMcC* were crossed as male parents onto *Sh bz-R* (null) females. Pericarps and embryos were removed from 20 mature F_1 (*Bz′/bz-R/bz-R*) kernels. The endosperms and aleurones were ground to a fine powder in a commercial coffee mill. Samples were extracted for 1 h at 4° C in high salt buffer [3 ml/gm dry weight], fractionated and dialyzed against a low salt buffer with 10% glycerol as described in Kuhn and Klein (1987). Extracts were quick frozen in a dry ice-ethanol bath and stored at −70° C.

Enzyme assays. UFGT levels were measured as described by Gerats et al. (1984) with modifications described in Kuhn and Klein (1987). Isoquercitrin was separated from quercetin according to the method of Dowe et al. (1989). Total protein content of the plant extracts was determined by colorimetric assay (Bio-Rad, Richmond, Calif) using Coomassie blue G-250 dye and bovine gamma globulin as a protein standard.

Results

Genetic and molecular analyses demonstrate the mutant phenotype of *bz-m4* D6856 is caused by a 6.7 kb *Ds* transposon in the untranslated leader region of the *Bz* locus (Klein et al. 1988). The 6.7 kb transposon has 2 kb *Ds* elements at both ends. These surround a duplication of *bz* 3′ flanking sequences. Further molecular characterization of this complex allele suggested that there might be additional rearrangements in the distal flanking sequences at the *bz* locus in *bz-m4* as compared with its wild-type progenitor. Hence, experiments were undertaken to map extensively the 3′ flanking sequences of *bz-m4* D6856 and revertant alleles. The genomic map of *bz-m4* D6856 is presented in Fig. 1 and supporting data are described below. The composition of this allele is complex. The restriction data are described in some detail; these data provide evidence for the genomic structures of *bz-m4* D6856 and revertants arising from transpositions of tagged *Ds* elements.

Wild-type 3′ flanking sequences are deleted in bz-m*4 D6856*

The plasmid pMBzPR11 corresponds to the farthest 3′ flanking sequences from the clone of the wild-type allele, *BzMcC* (Furtek 1986; Fig. 1). The probe pMBzPR11 did not hybridize to *bz-m4* D6856 DNA, indicating that the more distal 3′ flanking sequences from the *bz* locus have been deleted in this allele. Sequences complementary to the probe pMBzP11, which are immediately upstream of pMBzPR11 in the wild-type allele (Fig. 1), are present in *bz-m4* D6856 (Table 1). This suggests that one endpoint of the deletion in D6856 is within 3′ flanking sequences corresponding to pMBzP11. The approximate position at which the two alleles, *BzMcC* and *bz-m4* D6856, diverge is marked with an asterisk (Klein et al. 1988).

Bronze coding sequences are duplicated in bz-m*4 D6856*

*Sst*I digests of wild-type *BzMcC* and D6856 DNA were probed with a *Kpn*I-*Sst*I fragment of the coding sequence probe pMBzP17 (Fig. 2a, b). The probe hybridized to a 3.8 kb wild-type fragment (Fig. 2a, lane 1) and to two fragments, 10.5 and 6.9 kb, of *bz-m4* DNA (lane 2). The 10.5 kb fragment is that predicted from the structure of the genomic clone of D6856 (the 3.8 kb *Sst*I fragment + the 6.7 kb transposon; Klein et al. 1988). *Bz′* revertants (lanes 3,5) have both the restored 3.8 kb wild-type *Sst*I fragment and the 6.9 kb fragment. Genomic Southern data, summarized in Table 1, and densitometry measurements (data not shown) indicate that the 6.9 kb band represents a second copy of *Bz* coding and a third copy of the *bz* 3′ flanking sequences (Fig. 1). The duplicated sequences are 4.85 kb. Genetic evi-

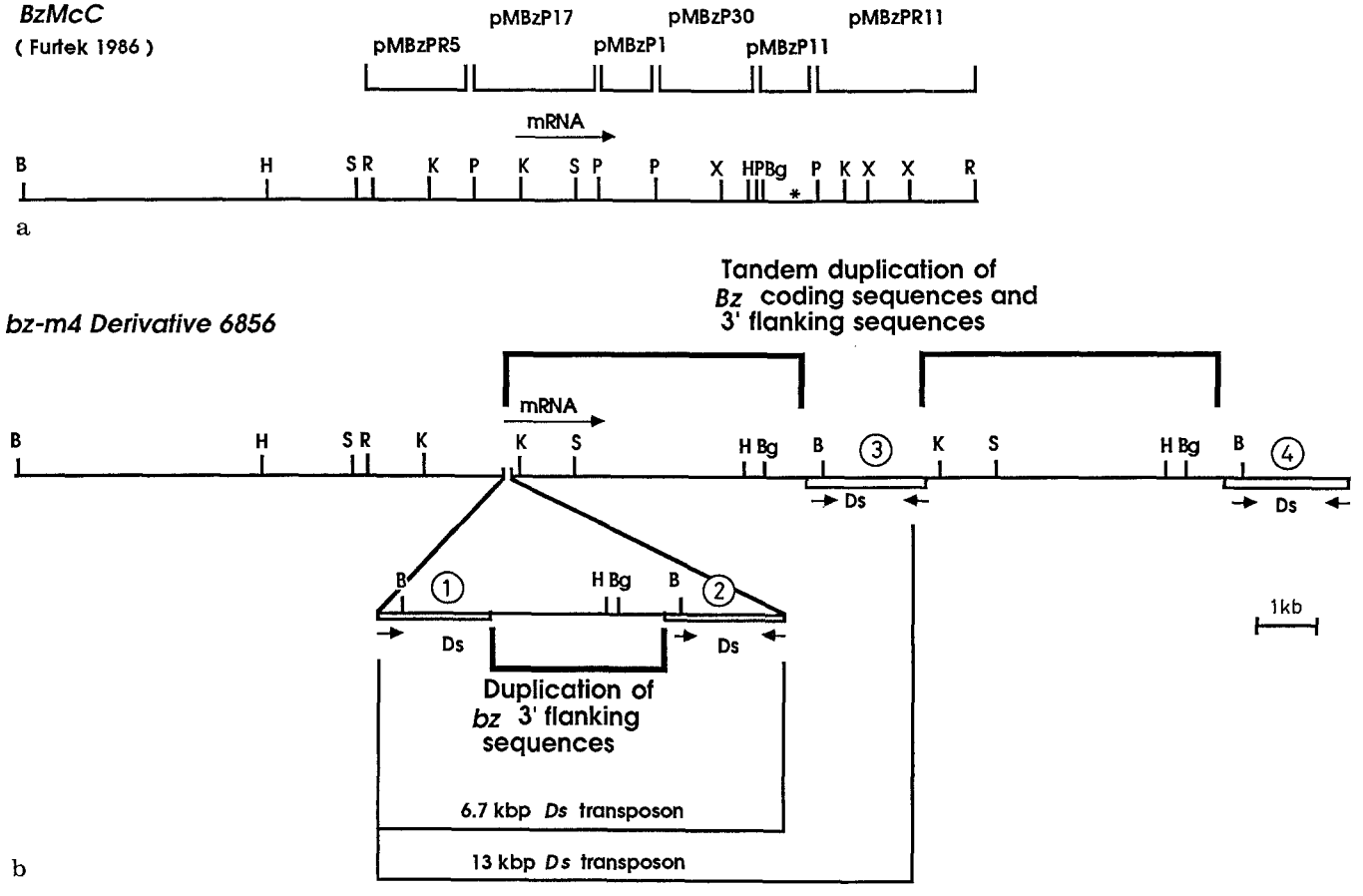

Fig. 1. Genomic restriction maps of *BzMcC* and *bz-m4* D6856. Subclones of pMBzR1, the *Eco*RI clone of the wild-type allele, are shown at the top of the figure. The relative position of the *BzMcC* mRNA transcript is indicated by a *large arrow*. The 5′ end of the map of *BzMcC* (Furtek 1986) is extended for comparison with *bz-m4* D6856. *Ds* elements in *bz-m4* are indicated by *open boxes*, numbered one through four. Inverted repeats are indicated by *small arrows*. Sequences to the right of the *asterisk* on the map of *BzMcC* are deleted from *bz-m4*. B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; *P*, *Pst*I; R, *Eco*RI; S, *Sst*I; X, *Xho*I

Table 1. Restriction fragments of genomic DNAs

Enzyme	Probe	Genotype	Observed fragment sizes (kb)
*Bam*HI	pMBzP30	*BzMcC*	17.5 (18.5)[a]
		bz-m4 D6856	6.8 (6.9)[b], 4.6 (4.8)
		Bz′:102, 104, 106, 110, 111, 112, 116, 117	11.5, 6.8, 4.6 (4.8)[b]
		Bz′:1, 103, 105, 106, 108, 109, 113, 115, 118	11.5, 6.7
		Bz′:107	11.5
		Bz′:114	11.5, 4.7, 3.7
	3′ *Kpn*I-*Pst*I of pMBzP17	*BzMcC*	13.5 (18.5)[a]
		bz-m4 D6856	6.7 (6.9)[b]
		Bz′:102, 103, 104, 106, 117	11.5, 6.7 (6.9)[b]
	pMBzP1	*BzMcC*	17.5 (18.5)[a]
		bz-m4 D6856	6.8 (6.9)[b]
		Bz′:105, 115, 116	11.5, 6.8
		Bz′:107	11.5
*Bgl*II + *Eco*RI	pMBzPR11	*BzMcC*	4.1 (3.4)[a]
		bz-m4 D6856	6.5 (6.8)[b]
		Bz′:105/*Bz′*:105′	6.5, 4.85
		Bz′:107	6.5
		Bz′:114	6.8
*Hin*dIII	*Kpn*I-*Sst*I of pMBzP17	*BzMcC*	7.8 (7.9)[a]
		bz-m4 D6856	7.0 (6.9)[b]
		Bz′:105	7.8, 7.0
		Bz′:107	7.8
		Bz′:114	7.8, 7.7
	pMBzP30	*BzMcC*	7.8 (7.9)[a]
		bz-m4 D6856	7.9 (6.9)[b], 7.0
		Bz′:1, 103, 105, 108, 109, 113, 115, 118	7.8, 7.0
		Bz′:107	7.8
		Bz′:114	7.8, 6.4
	pMBzP11	*Bz′*:1, 103, 108, 109, 113, 115, 118	6.9/7.1 (bands overlap)
*Hin*dIII	pMBzP11	*Bz′*:105/*Bz′*:105′	6.9/7.1, 4.9
	*Kpn*I-*Pst*I of pMBzPR5	*BzMcC*	7.9
		bz-m4 D6856	7.85
		Bz′:110	10
*Kpn*I	3′*Kpn*I-*Pst*I of pMBzP17	*BzMcC*	4.9 (5.6)[a]
		bz-m4 D6856	14, 7.2
		Bz′:105/*Bz′*:105′	14, 12, 7.2
		Bz′:107	14
		Bz′:114	18, 7.5
*Kpn*I + *Bam*HI	5′ *Pst*I-*Kpn*I of pMBzP17	*BzMcC*	1.6 (1.65)[a]
		bz-m4 D6856	2.1 (2.1)[b], 1.4 (1.6)[b]
		Bz′:102, 103, 104, 106, 110, 111, 112, 114, 116, 117	2.1, 1.6
		Bz′:107	1.6
Second blot			
*Kpn*I + *Bam*HI		*BzMcC*	1.75 (1.65)[a]
		bz-m4 D6856	2.3 (2.1)[b], 1.65 (1.61)
		Bz′:105, 107, 108, 109, 113, 114, 115, 118	2.25, 1.75
		Bz′:107	1.75
	*Kpn*I-*Sst*I of pMPzP17	*BzMcC*	17.5 (18.5)[a]
		bz-m4 D6856	6.8 (6.9)[b]
		Bz′:105, 115, 116	15, 6.8
		Bz′:114	15, 5.2
		Bz′:107	15
	*Eco*RI-*Kpn*I of pMBzPR5	Bz′:105	1.5
*Kpn*I + *Hin*dIII	3′*Kpn*I-*Pst*I of pMBzP17	*BzMcC*	3.8 (3.54)[a]
		bz-m4 D6856	3.8 (3.54)[b]
		Bz′:105	3.8
		Bz′:107	3.8
		Bz′:114	4.5, 3.8
	pMBzP11	*BzMcC*	1.7 (1.6)[a]
		bz-m4 D6856	7.1, 3.5 (3.0)[b]
		Bz′:105	7.1, 3.5
		Bz′:107	7.1
		Bz′:114	4.5, 3.5
	*Eco*RI-*Kpn*I of pMBzPR5	*Bz′*:105/*Bz′*:105′	1.5
*Kpn*I + *Sst*I	*Eco*RI-*Kpn*I of pMBzPR5	*Bz′*:105/*Bz′*:105′	1.25
		Bz′:110	8.0
*Kpn*I + *Eco*RI	*Eco*RI-*Kpn*I of pMBzPR5	*Bz′*:110	7.8
*Sst*I		*BzMcC*	3.6 (3.8)[a]
		bz-m4 D6856	11 (11.5)[b], 7.0 (6.9)[b]
		Bz′:102, 104, 105, 106, 111, 112, 116, 117	7.0, 3.6
		Bz′:110	9.7, 7.0
		Bz′:107	3.6 (3.8)[a]
		Bz′:114	7.0, 3.6 (3.8)[a]
	pMBzP30	*BzMcC*	4.6 (4.6)[a]
		bz-m4 D6856	11 (11.5)[b], 7.0 (6.9)
		Bz′:105/*Bz′*:105′	14.5, 12, 7.0
		Bz′:107	14.5
		Bz′:1, 103, 108, 109, 113, 115, 118	14.4, 7.0
		Bz′:114	19, 7
	*Kpn*I-*Pst*I of pMBzPR5	*BzMcC*	3.8
		bz-m4 D6856	10.5
		Bz′:110	10.5
*Sst*I-*Bam*HI	*Kpn*I-*Pst*I	*BzMcC*	3.8
		bz-m4 D6856	2.9
		Bz′:110	3.3

Representative blots supporting these data are shown in Figs. 2, 3, and 5

[a] Expected fragment sizes (in parentheses) for *BzMcC* digests based on Furtek (1986)

[b] Expected fragment sizes (in parentheses) for *bz-m4* D6856 digests based on Klein et al. (1988)

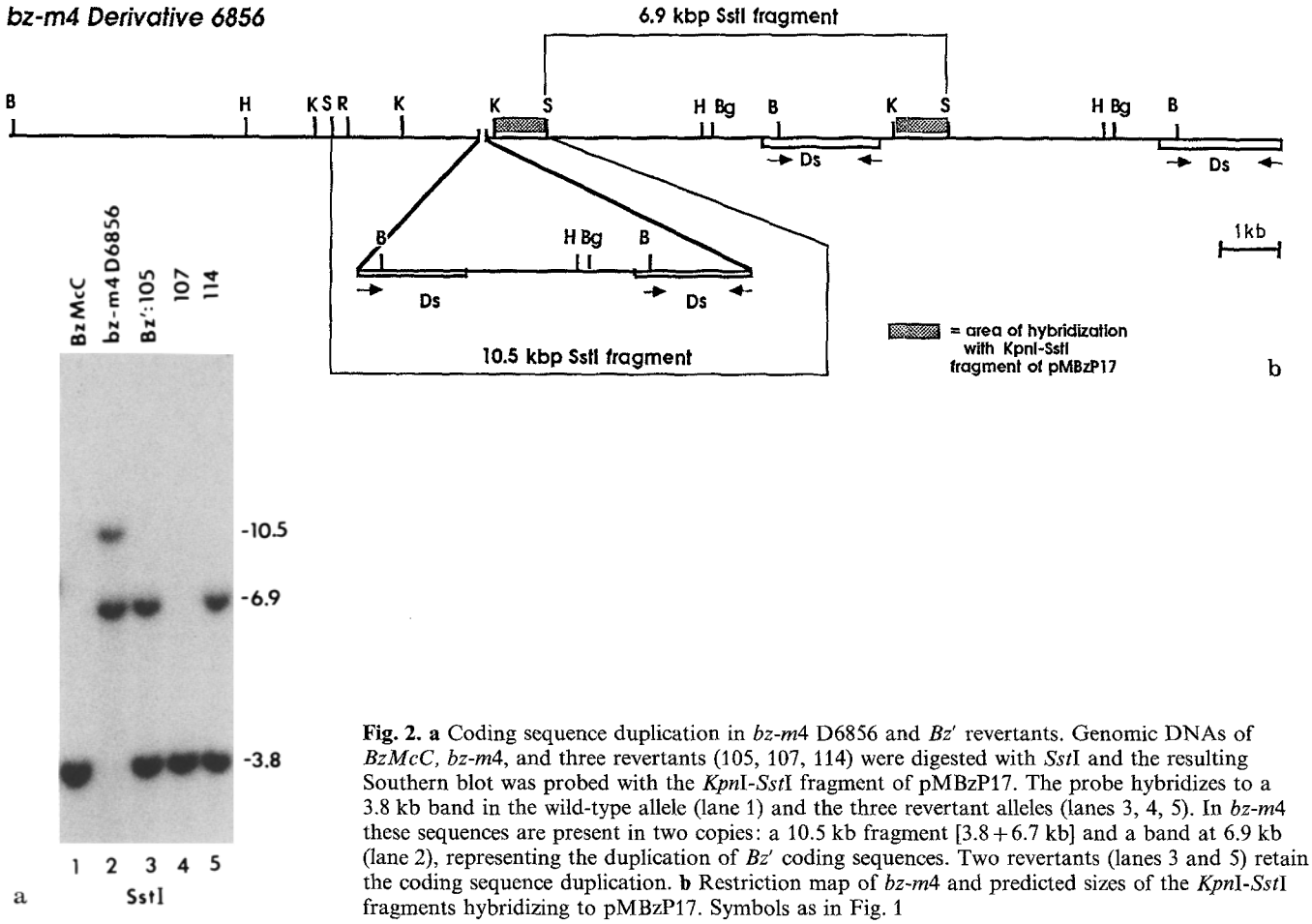

Fig. 2. a Coding sequence duplication in *bz-m4* D6856 and *Bz'* revertants. Genomic DNAs of *BzMcC*, *bz-m4*, and three revertants (105, 107, 114) were digested with *Sst*I and the resulting Southern blot was probed with the *Kpn*I-*Sst*I fragment of pMBzP17. The probe hybridizes to a 3.8 kb band in the wild-type allele (lane 1) and the three revertant alleles (lanes 3, 4, 5). In *bz-m4* these sequences are present in two copies: a 10.5 kb fragment [3.8 + 6.7 kb] and a band at 6.9 kb (lane 2), representing the duplication of *Bz'* coding sequences. Two revertants (lanes 3 and 5) retain the coding sequence duplication. **b** Restriction map of *bz-m4* and predicted sizes of the *Kpn*I-*Sst*I fragments hybridizing to pMBzP17. Symbols as in Fig. 1

dence, presented below, suggests that the remaining 2.05 kb is a third *Ds* element (total fragment size = 6.9 kb). Revertant *Bz'*:107 (lane 4) has a single wild-type size *Sst*I fragment. (The structure of this revertant will be described in more detail below.)

The duplication of Bz *coding sequences and 3' flanking sequences in* bz-m*4 D6856 is in direct tandem orientation*

In a direct tandem duplication, the distances between corresponding restriction sites in each copy will always be equal. This generalization should hold true for any restriction enzyme which cuts once within each copy of the tandem duplication. DNA from the *bz-m4* D6856 line was digested with *Kpn*I, *Sst*I or *Hin*dIII and probed with the coding sequences from pMBzP17 (Table 1). In each case the probe hybridized to two fragments, one corresponding in size to the wild-type fragment + the 6.7 kb transposon and one fragment which was 6.9 kb in size. The majority of the revertants also retain this 6.9 kb fragment. Similar data were obtained for *Bam*HI digests (see below). These data indicate that the duplication of *bz* coding, and a third copy of 3' flanking sequences are in direct tandem orientation in *bz-m4* D6856 (Fig. 1).

Excision of the 6.7 kb Ds *transposon in* Ac*-mediated reversion of* bz-m*4 D6856*

The line homozygous for *bz-m4* D6856 does not produce full purple revertants unless an active *Ac* has been introduced by appropriate crosses (A. Klein, unpublished observations). This *bz-m4* line was crossed to an *Ac* bearing strain and 17 new *Bz'* alleles were isolated from an F_2 population. The frequency of reversion to full color was 7.5×10^{-2}. *Bz'*:1 was obtained from Dr. Hugo Dooner (1981).

The 6.7 kb *Ds* transposon in *bz-m4* is inserted in what corresponds to +36 bp in the 5' untranslated leader region of the *Bronze* mRNA (Klein et al. 1988). The insertion site is within a 1.65 kb *Kpn*I fragment of the *BzMcC* allele; the corresponding *Kpn*I fragment of *bz-m4* is 8.35 kb (Fig. 1). Excision of the 6.7 kb *Ds* transposon (from the first *Ds* through the second *Ds*) from *bz-m4* would bring the *bz* upstream regulatory elements and the mRNA cap site into direct juxtaposition with the *Bz* coding sequences and restore the wild-type sized *Kpn*I fragment. All 18 full purple revertants examined have the wild-type sized 1.65 kb *Kpn*I fragment (data not shown). A 6.9 kb *Kpn*I fragment, representing the coding sequence duplication, is retained in 17 of the revertant alleles (all but *Bz'*:107; data described below).

Transposition of the tagged Ds *elements from* bz-m*4 D6856*

In *Bam*HI digests of *bz-m4* DNA, three fragments hybridize to the 3' flanking sequences probe, pMBzP30: a doublet of ~6.9 kb and a 4.8 kb fragment (Fig. 3a, lane 2; the high molecular weight band in this lane is due to partial digestion). The 4.8 kb band corresponds to most of the first *Ds*, the duplication of *bz* 3' flanking sequences within the

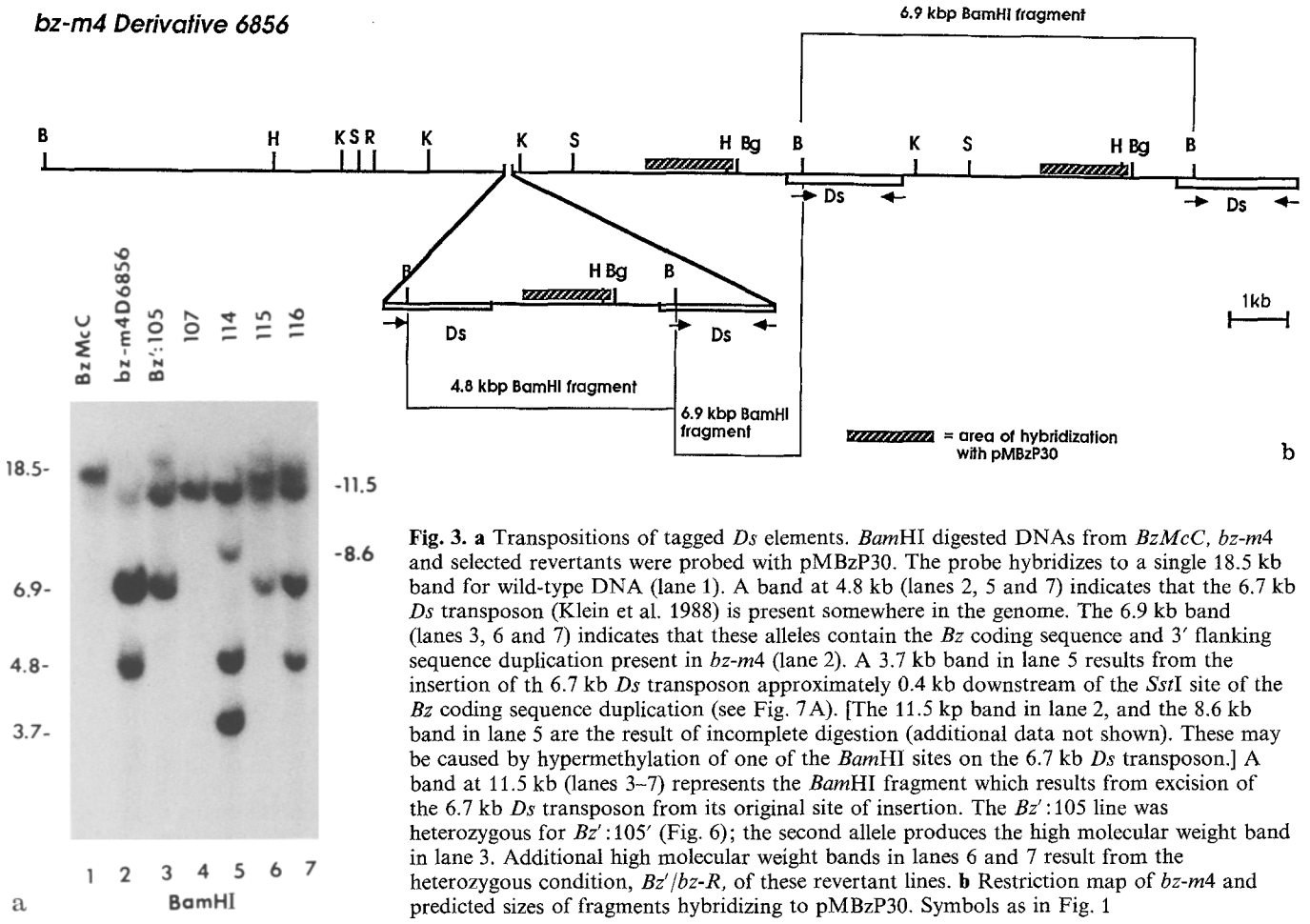

Fig. 3. a Transpositions of tagged *Ds* elements. *Bam*HI digested DNAs from *BzMcC*, *bz-m4* and selected revertants were probed with pMBzP30. The probe hybridizes to a single 18.5 kb band for wild-type DNA (lane 1). A band at 4.8 kb (lanes 2, 5 and 7) indicates that the 6.7 kb *Ds* transposon (Klein et al. 1988) is present somewhere in the genome. The 6.9 kb band (lanes 3, 6 and 7) indicates that these alleles contain the *Bz* coding sequence and 3′ flanking sequence duplication present in *bz-m4* (lane 2). A 3.7 kb band in lane 5 results from the insertion of th 6.7 kb *Ds* transposon approximately 0.4 kb downstream of the *Sst*I site of the *Bz* coding sequence duplication (see Fig. 7A). [The 11.5 kp band in lane 2, and the 8.6 kb band in lane 5 are the result of incomplete digestion (additional data not shown). These may be caused by hypermethylation of one of the *Bam*HI sites on the 6.7 kb *Ds* transposon.] A band at 11.5 kb (lanes 3–7) represents the *Bam*HI fragment which results from excision of the 6.7 kb *Ds* transposon from its original site of insertion. The *Bz′*:105 line was heterozygous for *Bz′*:105′ (Fig. 6); the second allele produces the high molecular weight band in lane 3. Additional high molecular weight bands in lanes 6 and 7 result from the heterozygous condition, *Bz′/bz-R*, of these revertant lines. **b** Restriction map of *bz-m4* and predicted sizes of fragments hybridizing to pMBzP30. Symbols as in Fig. 1

6.7 kb *Ds* transposon, through to the *Bam*HI site in the second *Ds* element (Fig. 3b). Excision of the 6.7 kb transposon, from *bz-m4*, produces an 11.5 kb *Bam*HI fragment (Fig. 3a, lanes 3, 4, 5, 6, and 7). If the transposon reinserts (e.g. transposes) in a chromatid which segregates with the new *Bz′* allele, the transposed *Ds* can be detected as a 4.8 kb *Bam*HI fragment. The transposed *Ds* was detected in 9 of the 18 *Bz′* lines (Table 2; Fig. 3a, lanes 5, and 7; other data not shown). Two of these were detected at new sites at the *bz* locus (*Bz′*:110 and *Bz′*:114; described below).

Structures of the Bz′ *revertants*

The structures of the *Bz′* revertant alleles were determined by genomic Southern mapping, summarized in Table 1, using probes from the various subclones of the wild-type *BzMcC* allele (Fig. 1 shows the subclones of *BzMcC*). The structure of *Bz′*:108 is shown in Fig. 4a and is typical of the structure of the majority of the revertants (except *Bz′*:105′, 107, 110 and 114 which are described below). *Sst*I digests of *Bz′*:108 DNA, probed with the *Kpn*I-*Sst*I fragment of pMBzP17 show 3.6 and 6.9 kb hybridizing fragments. These data indicate that the 6.7 kb *Ds* transposon has excised from the untranslated leader region restoring one copy of the *Bz* locus to normal regulation. The 6.9 kb fragment represents the second copy *Bz* coding sequences and is equivalent to that described for *bz-m4* (Fig. 1).

Excision of a 13 kb Ds *transposon,* Bz′:*107*

The structure of the full purple revertant allele *Bz′*:107 (Fig. 4b) provides evidence that there is a *Ds* element (labeled 3 in Fig. 1) between segments of the *Bz* duplication in *bz-m4*. The characteristic 6.9 kb *Bam*HI, *Hin*dIII, *Sst*I and *Kpn*I bands, which hybridize to the *Kpn*I-*Sst*I fragment of pMBzP17 and represent the coding sequence duplication, are absent in *Bz′*:107 (Fig. 3, lane 4, Fig. 5, lane 4, other data not shown). A single 3.8 kb *Sst*I fragment hybridizes to this probe (Fig. 2a, lane 4). This allele has arisen from an excision event in which the transposase has excised a segment beginning with the first *Ds* of the 6.7 kb transposon (Fig. 1) through to the third *Ds*. The excision restores the wild-type 1.65 kb *Kpn*I fragment at the 5′ end of the gene and leaves the revertant with only a single copy of *Bz* coding sequences.

An alternative explanation for the origin of *Bz′*:107 would be recombination between the *bz-R* allele and the second copy of *Bz* coding sequences in *bz-m4*. The *bz-R* mutation is caused by a 340 bp deletion of coding sequences (Ralston et al. 1988). This deletion is distal relative to the insertion site of the 6.7 kb transposon in *bz-m4*. A double recombinant, within the ~600 bp between *bz-R* and *bz-m4* inserts could produce a structure similar to *Bz′*:107. The probability of isolating a double recombinant in a small F_2 population (<5000 kernels) is very low. The 5′ and 3′ flanking regions of *bz-R* and *bz-m4* have two polymor-

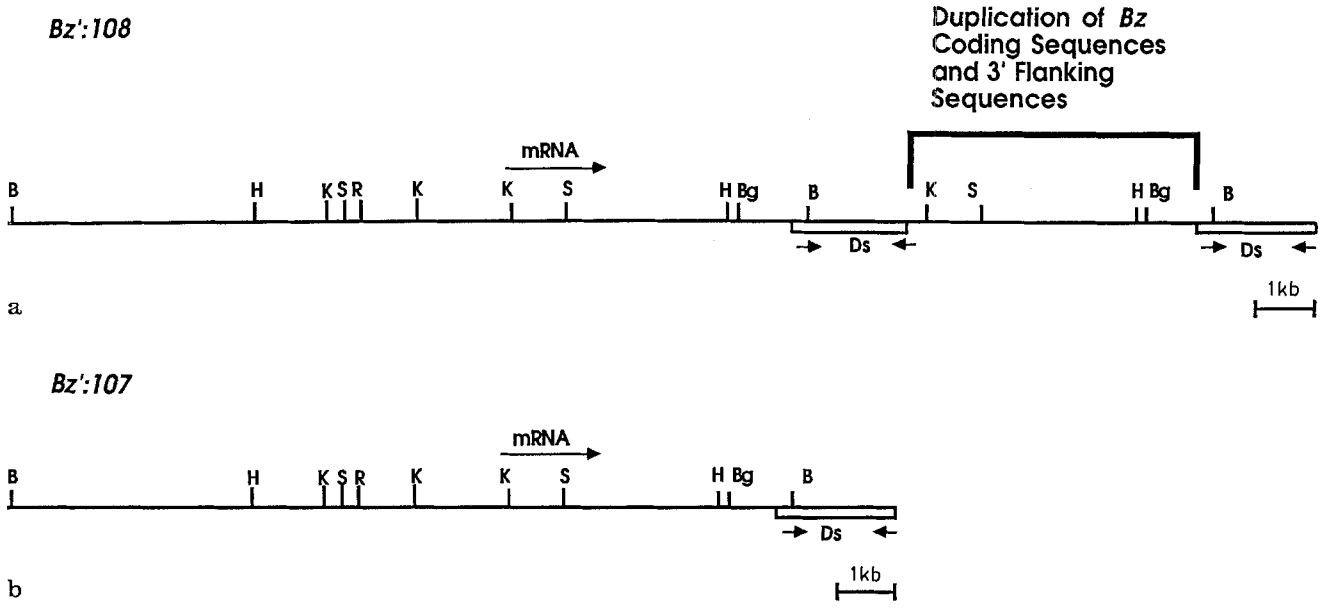

Fig. 4. **a** Excision of the 6.7 kb *Ds* transposon: genomic restriction map of *Bz'*:108. **b** Excision of the 13 kb *Ds* transposon: *Bz'*:107. Symbols as in Fig. 1

phisms which maybe used a molecular markers to distinguish recombinants from *Ac*-mediated revertants of *bz-m4*. The restriction map for the 5′ and 3′ flanking regions of *Bz'*:107 was similar to its progenitor *bz-m4* not that of *bz-R* (data not shown). Considering these data together, it is most probable that *Bz'*:107 arose from an *Ac*-mediated excision between the first *Ds* and a **third** *Ds* element in *bz-m4* D6856.

Molecular evidence for the fourth Ds *elements:* Bz′:*105 and* Bz′:*105′*

The *Bz'*:105 allele was selected in the F_2 seed from a cross between *bz-m4* D6856 and *bz-R Ac^wx-m9*. The revertant allele was carried through two additional generations of self pollination. The F_4 ear was full purple, indicating that the *bz-R* allele had segregated out in the previous meiosis. Sib kernels from the F_3 ear had *Bz* to *bz* sectors, characteristic of breakage-fusion-bridge cycles involving chromosome 9S. This observation suggests that an *Ac*-bearing chromosome was present at least in the F_3 generation. An immature tassel was harvested from a plant grown from F_4 seed. This plant was heterozygous for *Bz'*:105 (similar to *Bz'*:108) and *Bz'*:105′, an allele resulting from excision of the *Ds* element distal to the coding sequence and 3′ flanking sequence duplication.

Assuming *Bz'*:105 arose by excision and loss of the 6.7 kb *Ds* transposon, the *Kpn*I-*Sst*I fragment of pMBzP17 should hybridize to two *Kpn*I fragments of *Bz'*:105 DNA (Fig. 5b). A 6.9 kb band, representing the coding and 3′ flanking sequence duplication, and a 14 kb band, representing the most distal *Kpn*I fragment, are both observed in restriction digests of *Bz'*:105 DNA (Fig. 5a, lane 3). However, a third fragment of 12 kb also hybridizes to the probe. The intensities of the 12 and 14 kb bands are similar and both are less intense than the 6.9 kb band. *Sst*I digests probed with pMBzP30 produced 15.4, 12, and 7.0 kb fragments. A *Bgl*II-*Eco*RI digest of *Bz'*:105 DNA, probed with pMBzP11, produced a 6.9 kb doublet and an additional 4.85 kb band (Table 1). In each case the additional band from the *Bz'*:105′ DNA was approximately 2 kb smaller than the band which represents the most distal restriction fragment (Table 1). The *Ds* elements at the *Bz* locus in *bz-m4* D6856 are 2 kb in size.

These data suggest that the plant carrying the *Bz'*:105 allele was heterozygous with a *Bz'*:105′ allele (Fig. 6). We interpret these results such that the *Bz'*:105′ allele arose from an *Ac*-mediated excision event removing the most distal *Ds* element in a generation subsequent to that in which the initial reversion event occurred. *Bz'*:105′ is identical to *Bz'*:105 except that *Bz'*:105′ lacks the most distal *Ds* element (equivalent to the fourth *Ds*, Fig. 1).

Localized transposition of the 6.7 kb Ds *element from* bz-m*4*

The 6.7 kb transposon was found reinserted within a few kilobases of its original position in two of the *Bz'* revertants. Southern blots of *Bam*HI digests of *Bz'*:114 were hybridized to the probe pMBzP30 (Fig. 3a, b); sequences homologous to the probe pMBzP30 are present in three copies in this revertant with fragments at 11.5, 4.8, and 3.7 kb (Fig. 3a, lane 5). (Densitometry studies as well as other mapping data suggest that the 8.6 kb band which is also visible results from incomplete cutting of a *Bam*HI site and is essentially the 3.7 kb fragment plus the 4.8 kb fragment.) Unlike the other 16 *Bz'* alleles, there is no 6.9 kb fragment. This suggests that the *Ds* transposon re-inserted in the second copy of *Bz* coding and flanking sequences. *Hin*dIII digests of *Bz'*:114 DNA probed with pMBzP30 revealed a 5.9 band and a 7.9 kb band (Table 1). Densitometric analysis indicated that the 5.9 kb band contained two copies of pMBzP30 while the 7.9 kb band contained only one copy of pMBzP30 sequences (data not shown). These data indicate that the transposon has re-inserted in inverted orientation relative to *bz-m4*. Further evidence for the inverted orientation of the 6.7 kb transposon is that the probe

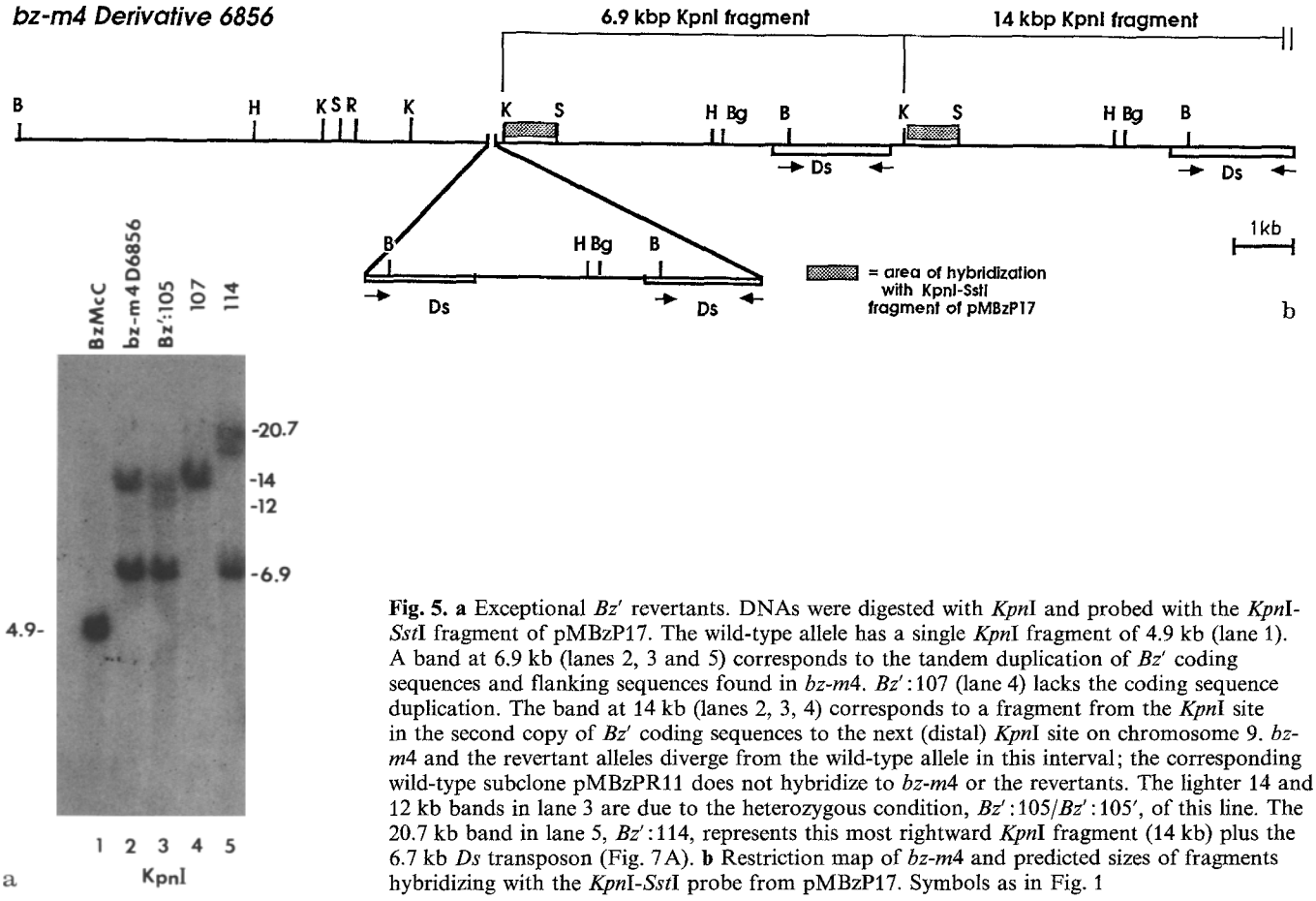

Fig. 5. a Exceptional *Bz'* revertants. DNAs were digested with *Kpn*I and probed with the *Kpn*I-*Sst*I fragment of pMBzP17. The wild-type allele has a single *Kpn*I fragment of 4.9 kb (lane 1). A band at 6.9 kb (lanes 2, 3 and 5) corresponds to the tandem duplication of *Bz'* coding sequences and flanking sequences found in *bz-m4*. *Bz'*:107 (lane 4) lacks the coding sequence duplication. The band at 14 kb (lanes 2, 3, 4) corresponds to a fragment from the *Kpn*I site in the second copy of *Bz'* coding sequences to the next (distal) *Kpn*I site on chromosome 9. *bz-m4* and the revertant alleles diverge from the wild-type allele in this interval; the corresponding wild-type subclone pMBzPR11 does not hybridize to *bz-m4* or the revertants. The lighter 14 and 12 kb bands in lane 3 are due to the heterozygous condition, *Bz'*:105/*Bz'*:105', of this line. The 20.7 kb band in lane 5, *Bz'*:114, represents this most rightward *Kpn*I fragment (14 kb) plus the 6.7 kb *Ds* transposon (Fig. 7A). **b** Restriction map of *bz-m4* and predicted sizes of fragments hybridizing with the *Kpn*I-*Sst*I probe from pMBzP17. Symbols as in Fig. 1

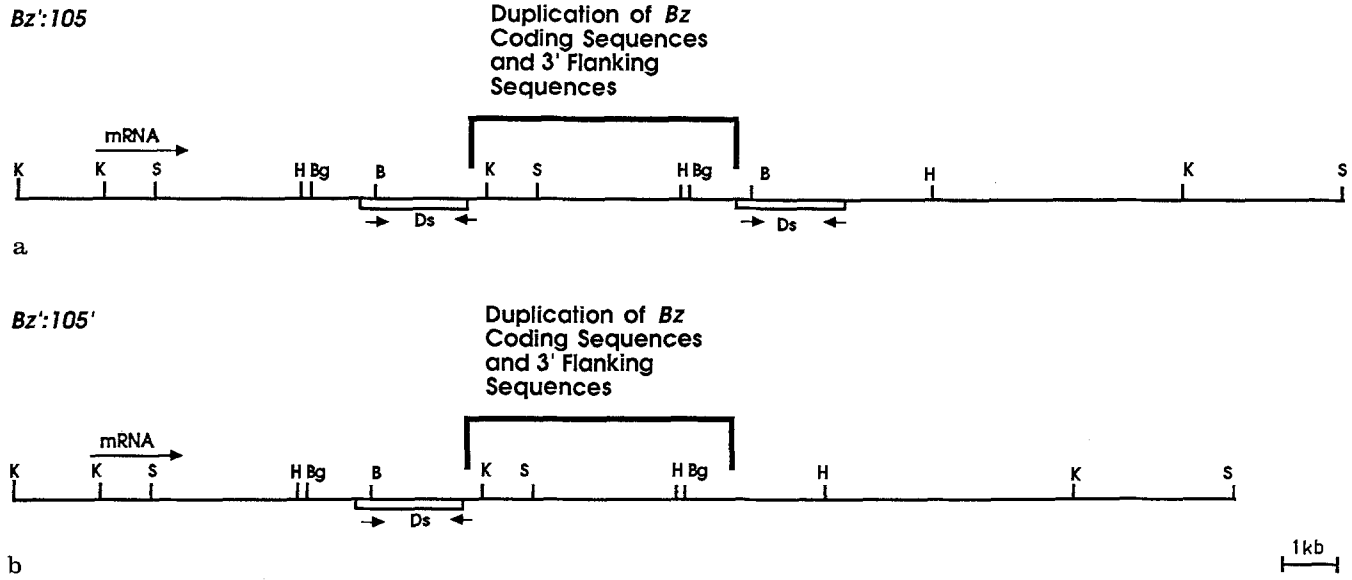

Fig. 6. a Distal 3' flanking restriction sites in *Bz'* revertants: *Bz'*:105. The order and spacing of these restriction sites were similar for *bz-m4* D6856 and the other *Bz'* revertants (data not shown). **b** Excision of the fourth *Ds* element: *Bz'*:105'. Symbols as in Fig. 1

pMBzP11 and the *Kpn*I-*Sst*I fragment of pMBzP17 both hybridize to a 4.6 kb *Kpn*I-*Hin*dIII fragment of *Bz'*:114 (Table 1). The probe pMBzP30 does not hybridize to this 4.6 kb *Kpn*I-*Hin*dIII fragment (Table 1). The genomic restriction map for *Bz'*:114 is shown in Fig. 7a.

The *Eco*RI-*Kpn*I fragment of pMBzPR5 hybridizes to a 7.8 kb *Eco*RI-*Kpn*I band in *Bz'*:110 DNA. pMBzP30 also hybridizes to this band (Table 1). These data localize the transposed *Ds* between the *Eco*RI and *Kpn*I sites immediately upstream in the 5' flanking sequences of the revertant.

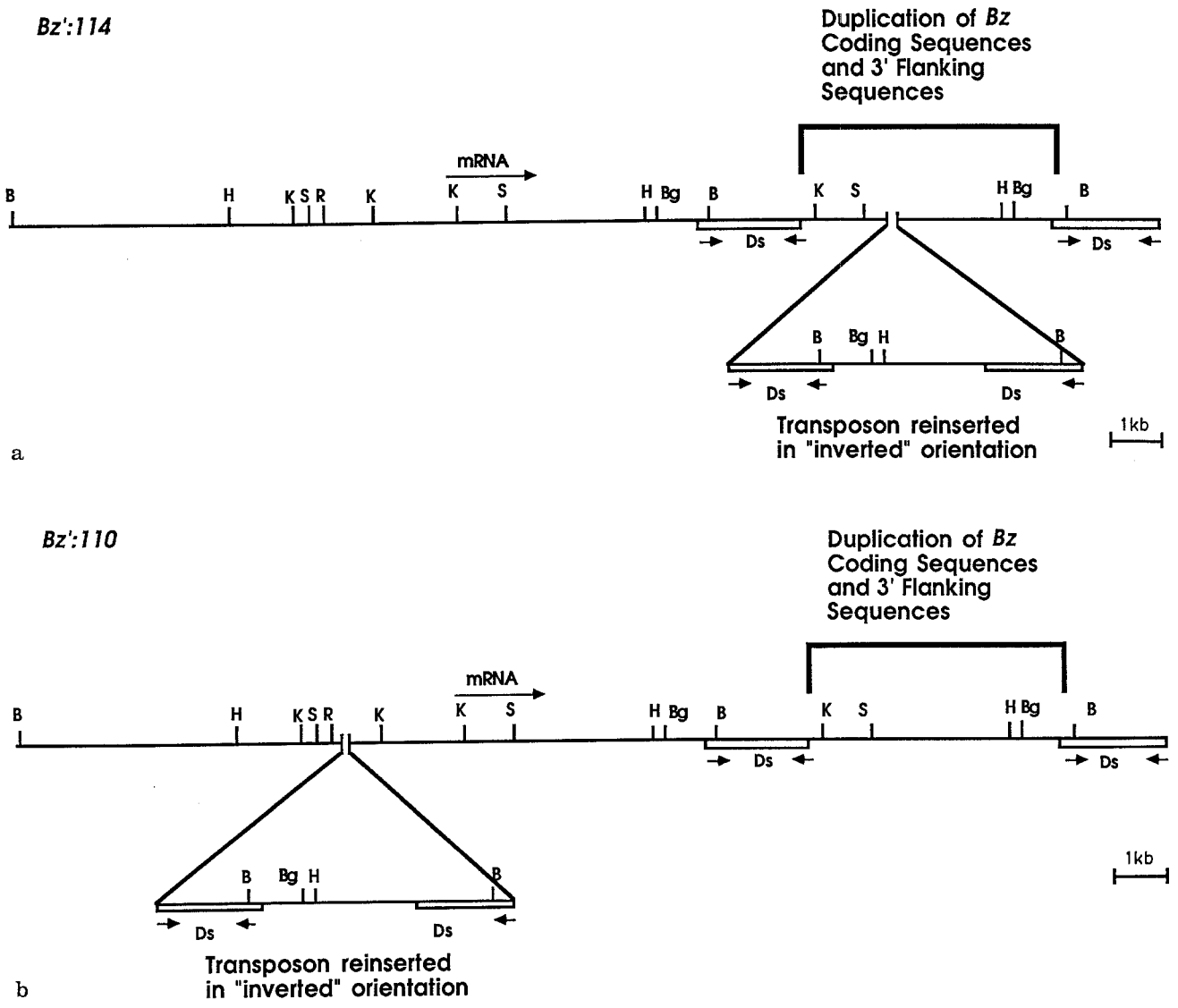

Fig. 7a and b. Localized transposition of the 6.7 kb *Ds*. Restriction maps of **a** *Bz'*:114 and **b** *Bz'*:110. Symbols as in Fig. 1

The *Kpn*I-*Pst*I fragment of pMPzPR5 hybridizes to a 10 kb *Hin*dIII fragment and a 3.3 kb *Sst*I-*Bam*HI fragment. This places the site of insertion 1.9 kb upstream of the mRNA cap site and in inverted orientation relative to *bz-m4* D6856 (Fig. 7b).

Excision of the complex Ds *transposons restores high UFGT activity*

The full purple revertants (*Bz'*:1, 102–118) resulted from excision of either the 6.7 or the 13 kb *Ds* transposons from *bz-m4* D6856. These events restore the 5′ untranslated leader region of the gene but presumably leave some footprint (part of the 8 bp target site duplication created when a *Ds* type element inserts) between the transcription and translation start sites. The *Bz'* revertant lines were crossed to a standard *bz-R* inbred to control for strain variation between the *Bz'* revertant lines and UFGT activity was measured from extracts of mature kernels (*Bz'*/*bz-R*/*bz-R*). All but one of the full purple revertants tested had UFGT expression levels that were equal to or greater than those of the wild-type allele (Table 2).

Table 2. Relative enzyme activities of *Bz'* alleles in mature kernels

Genotype	UFGT specific activity (mmol/h per mg protein equivalent)
BzMcC	0.073
Bz':1	0.079
Bz':102	0.091
Bz':103	0.001
Bz':104	0.075
Bz':105	0.071
Bz':106	0.082
Bz':107	0.092
Bz':108	0.083
Bz':109	0.111
Bz':110	0.076
Bz':111	0.096
Bz':112	0.104
Bz':113	0.095
Bz':114	0.114
Bz':115	0.100
Bz':116	0.087
Bz':117	0.095
Bz':118	0.082

UFGT activity for *Bz'*:103 was measured in extracts from kernels of a single ear, heterozygous *Bz'*:103/*bz-R*/*bz-R*, and was only 1.4% of wild-type activity. The enzyme activity measured for this revertant may be artificially low due to improper development of that particular ear. Plants grown in the summer of 1988 were damaged by hail storm in July and were particularly susceptible to insect and pathogen attacks. Extracts of kernels homozygous for *Bz'*:103, prepared the previous summer, had wild-type UFGT activity levels (data not shown).

Discussion

We have presented evidence that there are four *Ds* elements at the *bz* locus in the tissue-specific mutation *bz-m4* D6856. A partial duplication of 3′ flanking sequences is bracketed by single *Ds* elements (numbered 1 and 2 in Fig. 1) forming the 6.7 kb *Ds* transposon, described by Klein et al. (1988). The third *Ds* element follows the first copy of the *Bz* coding and another set of 3′ flanking sequences. Sequences beyond the third *Ds* are equivalent to a second copy of the coding region and a third copy of the 3′ flanking region of *bz*. A fourth *Ds* is present at the end of the entire complex.

BzMcC and *bz-m4* D6856 diverge at a point approximately 3 kb distal from the polyadenylation region (Fig. 1). DNA corresponding to the wild-type subclone pMBzPR11 does not hybridize to *bz-m4* or the *Bz'* revertants. Several restriction sites were mapped beyond the fourth *Ds* element; these are illustrated on the restriction maps for *Bz'*:105 and *Bz'*:105′ (Fig. 6). The 3′ flanking sites for D6856 and the other *Bz'* revertants are similar. In the origin of the *bz-m4* alleles, a deletion event from a *Ds* element distal to the *sh* locus may have fused a chromosomal segment from the interval between *I* and *Sh* on 9S to the distal flanking sequences of *bz-m4* (A. Klein, G. Roman, L. Paje-Manalo, unpublished observations).

Excision and transposition of Ds *elements*

Of the 17 full purple revertants which have resulted from excision of the 6.7 kb *Ds* transposon, 9 retain the *Ds* transposon in their genome (Table 3). The actual number of transpositions may have been higher. Some of the reinserted transposons may have been lost during post-excision meiotic segregation.

In *Bz'*:114, the 6.7 kb *Ds* transposon has reinserted 8.4 kb distal to its original position, in inverted orientation. In *Bz'*:110 the transposon has re-inserted 1.9 kb upstream of the wild-type mRNA cap site (proximal), also in inverted orientation. Using the genetic fine structure map of the *bz* locus (Dooner 1986) we can estimate the genetic distance for each transposition event. The calculated map distances should be taken as rough estimates, as the relationship between physical distance (kb) and genetic distance is probably not linear between loci (Dooner 1986). For *Bz'*:110, the 6.7 kb transposon has moved 0.14 map units proximal to its original insertion site. For *Bz'*:114, transposition was 0.6 map units distal from the original position of the 6.7 kb transposon. Accordingly our data, like that of Schwartz (1989), and Dooner and Belachew (1989) for the *Ac* element, show *Ds* elements regularly transpose over very short distances both proximal and distal to the original insertion sites. These results appear to contradict Greenblatt's (1984) model, that *Ac* transposes in a polar fashion during chromosome replication, inserting only into unreplicated segments of the chromosome.

Table 3. Excision and transposition of *Ds* transposons from *bz-m4* D6856

Revertant	Excision[a]	Transposon retained in genome
Bz':1, 103, 105, 108, 109, 113, 115, 118	6.7	No
Bz':102, 104, 106, 110, 111, 112, 114, 116, 117	6.7	Yes
Bz':107	13	No

[a] Size of *Ds* transposon in kb

Ds elements exist in high copy number (Nevers et al. 1986) in the maize genome. While there have been several reports of cloning genes tagged by *Ds* elements (Theres et al. 1987; Hake et al. 1989), in general it has not been practical to monitor transpositions of individual *Ds* elements (Kermicle et al. 1989). A large (>30 kb) *Ds* insertion has been described in the *sh-m5933* allele (Courage-Tebbe et al. 1983). In the *sh-m* allele, double *Ds* elements flank other genomic sequences, at least part of which are repetitive. Several *Sh* revertants were isolated but it is not clear whether the 30 kb *Ds* had transposed in these lines. The particular advantages provided by the *Ds* transposons in *bz-m4* D6856 are they are relatively small and contain low copy number *Bz* coding and/or flanking sequences. The characteristics of the *bz-m4* *Ds* elements allow their transposition to new sites to be followed easily.

The 6.7 kb insertion in *bz-m4* (Klein et al. 1988) alters the tissue specificity of *Bz* expression (Dooner 1981; L. Paje-Manalo, M. Dowe and A. Klein, unpublished observations). *Ac*-mediated excision of the 6.7 kb *Ds* transposon would bring the upstream regulatory region of the gene into direct juxtaposition with the first copy of *Bz* coding sequences. This should restore normal *Bz* gene activity. This type of revertant allele retains a duplication of the coding and 3′ flanking regions of the gene. *Ds* element insertions can be fully or partially transcribed (Döring et al. 1984), they may contribute translation start sites and splice junctions (Sachs et al. 1983), and they may be completely spliced out as an intron (Peacock et al. 1984; Wessler et al. 1987). If the 6.7 kb *Ds* transposon in *bz-m4* D6856 is merely spliced out of the primary transcript as an intron then it would be unlikely that the distal copy of the duplicated *Bz* coding sequences is transcribed, as this copy lacks the *Bz* promoter. If however, the *bz-m4* D6856 mRNA is being transcribed from a promoter in the second *Ds* (Fig. 1; Klein et al. 1988) then the other copy of *Bz* coding sequences may be transcribed as well since it is also preceded by a *Ds* element (labeled no. 3).

UFGT activity in the Bz′ *revertants*

Eighteen full purple revertants of *bz-m4* D6856 were examined; all but *Bz'*:107 resulted from excision of the 6.7 kb transposon (Table 3). Molecular and genetic evidence has been presented that *Bz'*:107 resulted from excision of the 13 kb *Ds* transposon. No reversion events involving excision of only single *Ds* elements or other combinations of the four *Ds* elements were detected in this study. Single *Ds* excisions may not confer a full purple phenotype and would not have been selected in the original screen.

Ac and *Ds* elements create an 8 bp target site duplication when they insert (Nevers et al. 1986). Excision can be precise, leaving behind only the 8 bp duplication or it can be imprecise, leaving behind a variable portion of the footprint at the insertion site. Presumably the *Bz'* revertants examined in this study have footprints. These footprints, which are in the 5' untranslated leader region of the *Bz* mRNA, appear not to have affected enzyme expression levels in the majority of revertants (Table 2). Dooner (1981) measured UFGT activity for five full purple revertants (*Bz'*:1, 2, 3, 4, 5) from *bz-m4*. His results were similar to ours in that each of *Bz'* alleles conditioned high enzymatic activity.

In summary, *Ds* elements have generated a tandem duplication of the *Bz* gene coding sequences in the *bz-m4* D6856 allele. This second copy of the gene may or may not be functional depending on the location of the transcription start site of the *bz-m4* D6856 transcript. The excision and transposition behavior of the 6.7 and the 13 kb *Ds* transposons from this locus is similar to what has been described for *Ac* elements. Reversion from *bz-m4* D6856 to a full purple phenotype occurs upon excision of either the 6.7 kb *Ds* transposon or the 13 kb *Ds* transposon from its original insertion site. Re-insertion of an excised 6.7 kb *Ds* transposon, like the autonomous element *Ac*, can be within a very short distance proximal or distal to its original position.

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