

Distribution of *Bs1* retrotransposons in *Zea* and related genera

S. I. Fuerstenberg* and M. A. Johns**

Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, USA

Received January 31, 1990; Accepted May 3, 1990

Communicated by A. R. Hallauer

Summary. Thirty-eight accessions from *Zea* and 20 accessions from related genera were probed for the presence of *Bs1*, a retrotransposon originally found in maize. All maize and teosinte plants tested show the presence of *Bs1* in one to five densely hybridizing bands. The mean copy numbers of *Bs1* elements among the maize and teosinte accessions were similar: 2.92 and 3.25, respectively, with no large differences between any subgroups. Most exotic maize samples exhibited two common bands of 7.8 kb and 4.7 kb. Section *Zea* teosintes (but not teosintes of section *Luxuriantes*) also show the presence of a common band of the same size as the smaller common band in maize. At reduced stringency, *Tripsacum dactyloides* exhibited a single hybridizing band at 6.9 kb. Results argue for the evolution of maize from a *mexicana* or *parviglumis* teosinte, and the evolution of the *Bs1* element within the tribe Andropogoneae. Additionally, recombinant inbred lines were probed for the presence of *Bs1*, in order to map the chromosomal locations of *Bs1* elements in four different maize lines. Two of the recombinant inbred parental lines had an element (*Bs1-F*) on chromosome 5, while the other two lines had an element (*Bs1-S*) on chromosome 8. Restriction site polymorphisms have apparently arisen in the vicinity of *Bs1-S* since its insertion. Segregation analysis of other lines was also performed; the data indicate that *Bs1* has the distribution expected of a transposable element, different locations in different lines, and not that of a fixed gene locus. However, the common bands in the *Zea mays* lines and the recombinant inbred data imply that *Bs1* is not highly mobile.

Key words: Retrotransposon – *Zea* – RFLP – *Bs1* – Transposable element

Introduction

The maize transposable element *Bs1* was first detected when it transposed into the *Adh1-S* gene in one of the progeny of a plant infected by barley stripe mosaic virus (Mottinger et al. 1984). Unlike most plant transposons, which are bounded by inverted repeats, *Bs1* is bounded by 302-bp terminal, direct repeats similar in structure to the LTRs of vertebrate retroviruses (Johns et al. 1985; Varmus 1983). Internally, the DNA sequence of *Bs1* contains a number of open reading frames, whose apparent amino acid sequence contains weak, but extensive, homologies to several retroviral proteins, including reverse transcriptase, protease, RNase H, and an endonuclease (Johns et al. 1989; Jin and Bennetzen 1989). These features allow *Bs1* to be classified as a retrotransposon, similar to *Ty* in yeast and the *copia*-like elements in *Drosophila* (Doolittle et al. 1989). Retrotransposons are rare in plants: only two other examples, in *Arabidopsis* (Voytas and Ausubel 1988) and in tobacco (Grandbastien et al. 1989), have been identified.

It has been proposed that retrotransposons are the degenerate remains of retroviruses, similar to the often defective, endogenous retroviruses found in vertebrates (Temin 1987). If *Bs1* were the result of a recent retroviral infection, then the taxonomic group that was initially infected (and its descendants) might contain many more copies of *Bs1* than other, closely related taxa. In a previous report (Johns et al. 1985), nine maize and two teosinte (*Z. mays mexicana*) lines were examined and found to contain one to five copies of *Bs1*. We have now

* Current address: Department of Plant Biology, University of Minnesota, 220 Biological Sciences Center, 1445 Gortner Ave, St. Paul, MN 55108, USA

** To whom correspondence should be addressed

Table 1. Lines analyzed

Line	Source	Collection
<i>Zea</i> section <i>Luxuriantes</i>		
1. <i>Z. perennis</i>	Collins	s.n. ^a
2. <i>Z. diploperennis</i>	Guzman	777
3. <i>Z. luxurians</i>	Iltis	G-5
4. <i>Z. luxurians</i>	Iltis	G-42
5. <i>Z. luxurians</i>	Iltis	G-38
<i>Zea</i> sections <i>Zea</i>		
<i>Z. mays</i>		
6. subsp. <i>parviglumis</i> var. <i>huehuetenangensis</i>	Iltis	G-120
7. subsp. <i>parviglumis</i> var. <i>parviglumis</i>	Iltis & Nee	1480
8. subsp. <i>parviglumis</i> var. <i>parviglumis</i>	Iltis & Cochrane	308
9. subsp. <i>parviglumis</i> var. <i>parviglumis</i>	Kato	K-77-13
10. subsp. <i>parviglumis</i> var. <i>parviglumis</i>	Benz	967
11. subsp. <i>parviglumis</i> var. <i>parviglumis</i>	Iltis & Doebley	8906
12. subsp. <i>parviglumis</i> var. <i>parviglumis</i>	Beadle	s.n.
13. subsp. <i>mexicana</i>	RPIS-A ^b	384062
14. subsp. <i>mexicana</i>	RPIS-A ^b	384074
15. subsp. <i>mexicana</i>	RPIS-A ^b	384069
16. subsp. <i>mexicana</i>	RPIS-A ^b	355921
17. subsp. <i>mexicana</i>	Doebley	625
18. subsp. <i>mexicana</i>	Doebley	642
19. subsp. <i>mexicana</i>	Doebley	481
20. subsp. <i>mexicana</i>	Beadle	s.n.
21. subsp. <i>mexicana</i> subsp. <i>mays</i>	Doebley	11066
22. Lady Finger Pop	MGC ^c	77-099
23. Knobless Wilbur's Flint	MGC ^c	81-1663
24. Tama Flint	MGC ^c	82-18964
25. Papago Flour Corn	MGC ^c	81-1588-3
26. Hulless Pop	MGC ^c	81-1641
27. Super Gold Pop	MGC ^c	82-7594
28. Tama Flint Knobless	MGC ^c	82-759-4
29. Black Mex Sweet W/B's	MGC ^c	84-675
30. Gourdseed	MGC ^c	81-1668
31. Maize Chapalote	MGC ^c	83-921-1
32. Ohio Yellow Pop	MGC ^c	81-1634
33. Smutnose	Doebley	222490
34. Shoepeg	Buckholt	s.n.
35. Hickory King	Sisco	84: 26
36. B73	Lifaco	s.n.
37. Mo17	Lifaco	s.n.
38. 5446	Freeling	s.n.
Other Andropogoneae ^d		
<i>Tripsacum dactyloides</i>		
39. 1008	Dewald	WW-1008
40. 1149	Dewald	WW-1149
41. 1152	Dewald	WW-1152
42. 1170	Dewald	WW-1170
43. 1181	Dewald	WW-1181
44. 1204	Dewald	WW-1204
45. 1241	Dewald	WW-1241
46. 1318	Dewald	WW-1318
47. 1582	Dewald	WW-1582

Table 1. (continued)

Line	Source	Collection
48. <i>Schizachyrium scoparium</i>	Pohl	s.n.
49. <i>Vetivera zizanioides</i>	Pohl	s.n.
50. <i>Cymbopogon citratus</i>	Pohl	s.n.
51. <i>Miscanthus sinensis</i>	Pohl	s.n.
52. <i>Elyonurus tripsacoides</i>	Doebley	646
53. <i>Sorghum bicolor</i>	Doebley	s.n.
Other Gramineae ^d		
54. <i>Hordeum vulgare</i> var. <i>vantage</i>	Zinnen	s.n.
55. <i>Avena fatua</i> var. <i>sativa</i>	Zinnen	s.n.
56. <i>Secale cereale</i>	Zinnen	s.n.
57. <i>Bambuseae</i> sp.	Zinnen	s.n.
58. <i>Chionachne koenigii</i>	Pohl	s.n.

^a Sine numero – not numbered^b Regional Plant Introduction Station-Ames^c Maize Genetics Cooperative^d According to Gould and Shaw (1968) and Hitchcock (1935)

extended this study to cover all of the known taxa of the genus *Zea* and some other species of Andropogoneae.

Previous evidence suggested that *Bs1* is not highly mobile in maize. No alterations in bands on Southern blots were seen among representatives of several generations of 1s2p maize or in other maize plants, both mutant and nonmutant, generated by barley stripe mosaic virus infection. Thus, the movement of *Bs1* into *Adh1* could have been a unique event. To find evidence of other *Bs1* transpositions, we mapped the positions of *Bs1* elements in several lines. Their independent positions suggest that *Bs1* elements are, in fact, transposable and not confined to fixed locations.

Materials and methods

Plant material

The genus *Zea* is composed of two sections (Iltis and Doebley 1980). Section *Zea* contains one species, *Z. mays*, which encompasses three subspecies, *Z. m. mays*, *Z. M. mexicana*, and *Z. m. Parviglumis*. Section *Luxuriantes* contains three species, *Z. perennis*, *Z. diploperennis*, and *Z. luxurians*. The terms "maize" and "exotics" refer to *Z. m. mays*, characterized by polystichous ears; all other *Zea* are referred to as teosintes, which have distichous ears. The 38 examples of *Zea* selected are representative of the genus, and include 5 lines from section *Luxuriantes* and 33 lines from section *Zea*, including 7 from ssp. *parviglumis*, 9 from ssp. *mexicana*, and 17 from ssp. *mays*. Twenty lines from other genera, including 9 of *Tripsacum dactyloides*, 5 other members of the tribe Andropogoneae, and 6 other Gramineae, were included to determine the extent of *Bs1* distribution in less closely related taxa. 5446 is the maize strain homozygous for the *Bs1* insertion in the *Adh1* genes (Mottlinger et al. 1984). Mut-bz is a line that contains a recessive *bronze* allele that is unstable in the presence of the *Mut* element (Rhoades and Dempsey 1982). *Mut* is not present in this line. A list of the lines analyzed and their origins is given in Table 1.

Recombinant-inbred stocks and RFLP analysis

Recombinant-inbred (RI) lines COXTx and TXCM were obtained from B. Burr and have been described elsewhere (Burr et al. 1988; Stuber and Edwards 1986). COXTx was derived from the inbred lines CO159 and Tx303, and TXCM was derived from T232 and CM37. Data obtained from the RFLP analysis on the RI lines were sent to B. Burr and analyzed using a FORTRAN program that compares distribution patterns of new probes to the distribution pattern of previously mapped loci.

DNA preparation, digestion, and genomic blot analysis

DNA was isolated from the freeze-dried tissue of 6- to 8-week-old seedlings by a modification of the method of Saghai-Marof et al. (1984).

Restriction enzymes were purchased from Bethesda Research Laboratories or International Biotechnology, Inc., and 10- μ g samples of DNA were digested with 50 units of enzyme for 4 h, according to the manufacturer's instructions. The digested DNA samples were electrophoresed through 0.7% agarose gels using $1 \times$ TBE (Maniatis et al. 1982) running buffer. DNA fragments in the gels were dephosphorylated in 0.25 M HCl, denatured in alkali (Kochetkov and Budovski 1972), neutralized, then transferred to nylon membranes (Amersham Hybond-N) according to Southern (1975). After drying, the filters were prehybridized for 2 h at 42°C in a solution of $6 \times$ SSPE, $10 \times$ Denhardt's solution, 0.5% SDS, and 50 μ g/ml salmon sperm DNA. Prehybridization buffer was then decanted and a hybridization buffer of $6 \times$ SSPE (pH 7.4), 0.5% SDS, 50% formamide, and 50 μ g/ml salmon sperm DNA was added. DNA fragments to be used as probes were radiolabeled with 32 P-dATP and 32 P-dCTP (ICN), using the random priming method of Feinberg and Vogelstein (1982). The probe used (probe 20) is a 550-bp SalI-SphI fragment of the insert in pK18, which has been described elsewhere (Johns et al. 1985) and is a fragment of *Bs1* internal to the retrotransposon LTRs. Denatured probe was added to the hybridization buffer to a concentration of 10^8 – 10^9 cpm/ μ g, and hybridizations were carried out at 42°C overnight. Hybridized filters were washed twice in $6 \times$ SSPE/0.2% SDS for 15 min at room temperature, twice in $1 \times$ SSPE/0.75% SDS for 15 min at 42°C, and once in either $0.1 \times$ SSPE/0.75% SDS (low stringency wash) or $0.05 \times$ SSPE/0.15% SDS (high stringency wash) for 30 min at 65°C. Hybridized filters were exposed to X-ray film (Kodak XAR-5) at -70°C for 3 days using Dupont Cronex intensifier screens.

Results

Copy number

A representative group of 16 *Zea* DNA samples was digested with EcoRI, EcoRV, DraI, and HindIII (none of which cut within the probe), and subjected to Southern blot analysis. Analysis of variance on the number of bands produced by each enzyme in each line showed no significant differences between the enzymes. For this reason, the rest of the work was carried out using EcoRI only, and the number of bands was considered to be equal to the copy number of *Bs1* elements.

All members of the genus *Zea* contained copies of *Bs1*, in approximately equal numbers. *Z. m. mays* accessions showed the presence of *Bs1* in one to five strongly

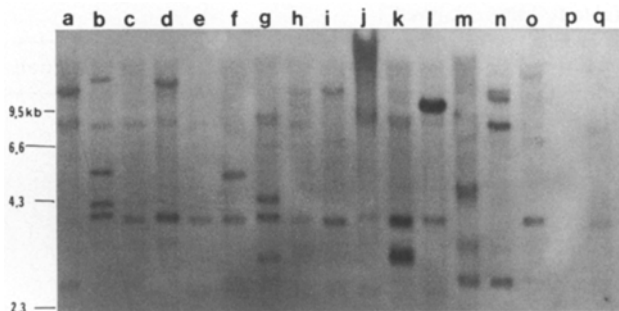


Fig. 1. Southern hybridization of exotic maize lines. Common bands can be seen at 7.8 and 4.7 kb. Each lane was digested with EcoRI and probed with an internal fragment of *Bs1* (probe 20). Also shown are the positions of HindIII-digested lambda DNA fragments used as molecular weight standards. *a* B73; *b* 5446; *c* Mo17; *d* Lady Finger Pop; *e* Knobless Wilbur's Flint; *f* Tama Flint; *g* Papago Flour; *h* Hulless Pop; *i* Supergold Pop; *j* Tama Flint Knobless; *k* Black Mexican Sweet; *l* Gourdseed; *m* Chapalote; *n* Ohio Yellow Pop; *o* Smutnose; *p* Shoepeg; *q* Hickory King

Table 2. Copy number summary

	Total	Exotics	Teosintes (com- bined)	Teosintes <i>Luxurian- tes</i>	Teosintes <i>Zea</i>
Plants examined	33	13	20	6	14
Total bands	103	38	65	18	47
Mean	3.12	2.92	3.25	3.00	3.36
s ²	0.80	1.24	0.51	0.00	0.71

hybridizing bands (Fig. 1). The mean copy number in these lines was 2.92 with a variance of 1.24 (Table 2). The teosinte accessions had a very similar copy number, 3.25 ± 0.51 , with little difference between section *Zea* and section *Luxuriantes*. The means were not strictly comparable, since the teosinte samples represented accessions from the wild, and the maize samples were partly or completely inbred lines. Therefore, the teosinte and maize samples had different and unknown levels of heterozygosity.

All *T. dactyloides* lines surveyed showed the presence of one hybridizing band at 6.9 kb, but only under low stringency wash conditions (Fig. 3). The number of *Bs1* bands in *Zea* samples did not change when low stringency wash conditions were used. A further decrease in the wash stringency did not increase the number or the intensity of the *Bs1* bands in either *Tripsacum* or *Zea*. Among the other members of the tribe Andropogoneae tested (Table 1), only *Schizacrium scoparius* and *Vetivera zizanioides* showed the presence of weakly hybridizing *Bs1* bands when washed at low stringency. No other Andropogoneae or members of the Gramineae showed the presence of *Bs1*.

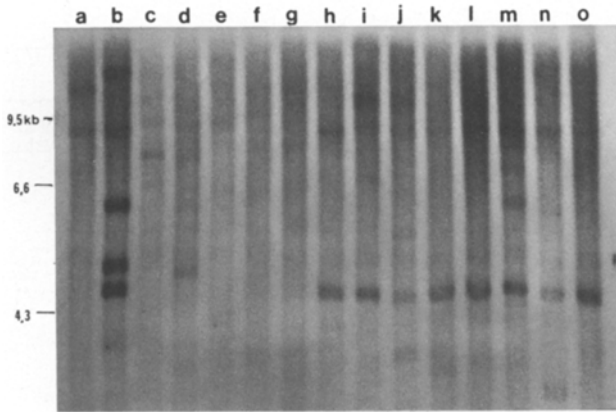


Fig. 2. Southern hybridization of teosinte lines. Each lane was digested with EcoRI and probed with an internal fragment of *Bs1* (probe 20). Also shown are the positions in HindIII-digested bacteriophage lambda DNA fragments used as molecular weight standards. The numbers following the line names refer to the identification numbers in Table 1. *a* B73; *b* 5446; *c* *Z. perennis*-1; *d* *Z. diploperennis*-2; *e* *Z. luxurians*-3; *f* *Z. luxurians*-4; *g* *Z. luxurians*-5; *h* *Z. mays parviglumis* var. *huethuetenangesis*-6; *i* *Z. m. parviglumis* var. *parviglumis*-7; *j* *Z. m. parviglumis* var. *parviglumis*-8; *k* *Z. m. parviglumis* var. *parviglumis*-9; *l* *Z. m. mexicana*-13; *m* *Z. m. mexicana*-14; *n* *Z. m. mexicana*-15; *o* *Z. m. mexicana*-16

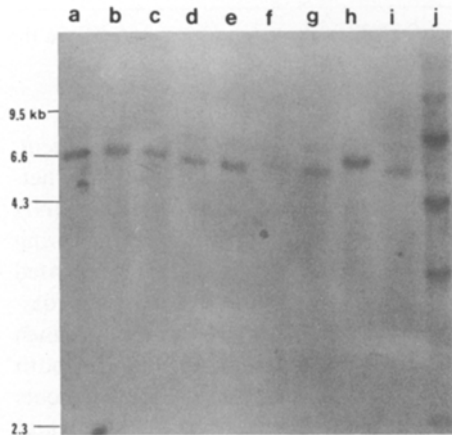


Fig. 3. Southern hybridization of *T. dactyloides* washed under low stringency conditions. The same blot washed using the normal high stringency conditions showed no hybridization except in lane (*j*), which showed the same bands visible here. *a* *T. dactyloides*-1008; *b* 1149; *c* 1152; *d* 1170; *e* 1181; *f* 1204; *g* 1241; *h* 1318; *i* 1582; *j* *Z. diploperennis*

Common bands

We noted the presence of a common band in most of the *Z. mays* plants, both maize and teosinte, at 4.7 kb (Figs. 1 and 2). Most of the maize accessions (but not the teosintes) also contained a common band at 7.8 kb. These bands were not seen in any of the *Luxuriantes* teosintes. Bands of a common size could be due to *Bs1* elements in a common position in all of the plants, but

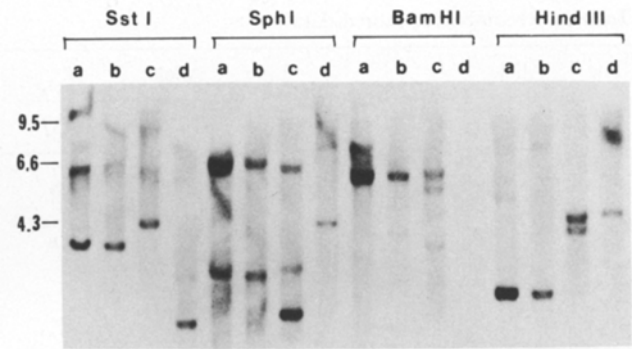


Fig. 4. Recombinant-inbred parental lines digested with different restriction enzymes. Note the common bands for CO159 and CM37, and the different band sizes for TX303 and T232. *a* CM37; *b* CO159; *c* T232; *d* TX303

common bands could also be generated by elements at different positions that happened to be flanked by EcoRI sites at similar distances apart. To show that the common bands were not artifactual or coincidental, DNA samples from these plants were digested with HindIII. Common bands appeared in the same plants with this enzyme as well, implying that a *Bs1* element exists in a common position in most *Z. mays* lines.

Mapping

Each of the parents from both RI lines, TXCM and COXTx, showed a single *Bs1* band when cut with EcoRI. Both RI lines were polymorphic for the size of the EcoRI band, allowing two *Bs1* elements to be mapped by comparison to data from previously mapped loci (Table 3). The *Bs1* elements in the four parental lines mapped to two distinct loci, allowing us to designate two separate elements, *Bs1-S* and *Bs1-F*. The *Bs1-F* element was found in the CO159 and CM37 parentals. Both of the parental bands for this element were the same size with EcoRI, approximately 2.3 kb, and no length polymorphisms were seen for *Bs1-F* with any of five restriction enzymes tested (Fig. 4). *Bs1-F* mapped to chromosome 5 between loci 7.58 and 6.10. The *Bs1-S* element was the slower-moving band found in TX303 and T232, and mapped to chromosome 8, closely linked to locus *Mdh1*, probably between *Mdh1* and 10.39. Although the *Bs1* elements in these two lines mapped to the same location, restriction fragment length differences were found with three out of five different restriction enzymes (Fig. 4). These length differences might be due to slightly different insertion sites or, alternatively, restriction site variations might have arisen in the vicinity of this site in the time since *Bs1* inserted in an ancestor of both lines. Since retrotransposons are thought to transpose through an RNA intermediate (Boeke et al. 1985) and to insert at random locations (Montgomery et al. 1987), we favor the latter hypothesis. The observed 1:1:1:1 ratio of *Bs1-S*: *Bs1-F*:

Table 3. Recombinant inbred data

Line	Allele		Line	Allele		Line	Allele		Line	Allele	
	T232	CM37		T232	CM37		TX303	CO159		TX303	CO159
2	1	1	32	1	2	58	1	2	83	2	1
4	1	1	33	1	2	59	1	1	84	2	2
6	1	1	34	1	2	60	1	1	85	2	2
7	1	1	35	2	1	61	2	1	86	1	2
9	2	1	36	1	1	62	2	1	87	1	1
11	1	1	37	1	2	63	1	1	88	1	1
12	2	1	38	1	1	64	1	2	89	2	1
13	2	1	39	1	1	66	1	2	90	1	2
14	1	1	40	2	2	67	2	1	91	1	1
15	2	1	41	1	2	68	1	1	92	1	2
16	2	1	42	2	2	69	2	1	93	1	2
17	2	1	43	1	2	71	1	2	94	2	2
18	2	1	44	2	1	72	2	2	95	1	1
19	1	1	45	2	1	73	2	2	96	1	2
20	2	2	46	2	1	75	1	1	97	1	2
21	1	2	47	1	1	76	1	2	98	1	2
22	2	2	48	2	2	77	2	1	99	2	2
23	1	2	49	1	1	78	1	1	100	1	1
24	1	1	51	1	2	80	1	1	102	1	1
25	1	2	53	1	1	81	2	2	103	1	1
26	2	1	54	1	1	82	1	1			
28	2	2	55	1	2						
29	2	1	56	2	2						
31	2	2	57	2	2						

A “1” in the T232 and CO159 columns indicates the presence of the parental *Bs1* element; a “2” indicates its absence. A “1” in the CM37 and TX303 columns indicates the absence of the parental *Bs1* element; a “2” indicates its presence

Table 4. Linkage test data and analysis

Mut-bz/B73 × 5446	Mut-bz/B73 × Mo17	Pooled data (Mut-bz/B73)		
Mut-bz bands	8	Mut-bz-bands	5	13
B73 bands	7	B73 bands	6	13
Mut-bz & B73	4	Mut-bz & B73	5	9
5446 only	6	Mo17 only	6	12
$\chi^2 = 1.696$	$\chi^2 = 0.182$	$\chi^2 = 0.915$		
$df = 3$	$df = 3$	$df = 3$		
$0.50 < P < 0.75$	$0.975 < P < 0.99$	$0.75 < P < 0.90$		
Mut-bz/Mo17 × 5446	Mut-bz/Mo17 × B73	Pooled data (Mut-bz/Mo17)		
Mut-bz bands	7	Mut-bz-bands	10	17
Mo17 bands	6	Mo17 bands	7	13
Mut-bz & Mo17	5	Mut-bz & Mo17	9	14
5446 only	4	B73 only	8	12
$\chi^2 = 0.909$	$\chi^2 = 0.588$	$\chi^2 = 1.000$		
$df = 3$	$df = 3$	$df = 3$		
$0.75 < P < 0.90$	$0.75 < P < 0.90$	$0.75 < P < 0.90$		

both *Bs1-S* and *Bs1-F*: neither *Bs1-S* or *Bs1-F* (25:19:20:24) confirmed that *Bs1-S* and *Bs1-F* are unlinked.

Additional information about the location of *Bs1* elements relative to one another was obtained by examin-

ing lines Mo17, B73, and Mut-bz. For this experiment, either Mo17 or B73 was crossed to Mut-bz. These heterozygotes were then crossed to a tester line and DNA was extracted from the resulting offspring. The offspring all contained *Bs1* bands from the tester and segregated for bands from the two original parents. For both crosses, Mo17/Mut-bz and B73/Mut-bz, the parental bands segregated in a 1:1:1:1 ratio of parent A: parent B: both parents: neither parent, implying that the *Bs1* elements are located in different, unlinked positions in Mo17 and Mut-bz, and in B73 and Mut-bz (Table 4). We were able to score only one band in B73 and Mo17, but two bands were scorable from the Mut-bz parent. These two bands always appeared together. Analysis of the same lines with HindIII and BamHI showed only a single band from Mut-bz. Thus, it is likely that Mut-bz contains an unusual *Bs1* element with an EcoRI site in the probed region, rather than two closely linked *Bs1* elements.

Discussion

The copy number of *Bs1* is low in all the taxa of genus *Zea*. We have found no examples of taxonomic groups with either exceptionally high or exceptionally low copy numbers. These data suggest that *Bs1* is not the degener-

ate remainder of a recent retroviral infection in some subgroup of *Zea*. On the contrary, the discovery of *Bs1*-like sequences in *Tripsacum* and some of the other Andropogoneae suggests that *Bs1* is a long-term resident of this tribe's genome.

Several possibilities might be invoked to explain the relatively low copy number of *Bs1*. One possible explanation stems from the observation that *Bs1* rarely transposes, and thus rarely has a chance to increase its copy number. Segregation analysis and recombinant-inbred mapping show that *Bs1* elements are in different places in different lines. This is the distribution expected of a transposable element, as opposed to a fixed gene locus. However, among the four recombinant-inbred parental lines, only two independent *Bs1* sites were found. Also, the existence of common bands in most of the *Z. mays* lines, both maize and teosinte, suggests that at least some of the *Bs1* elements have been stationary since before the differentiation of maize from teosinte. However, it is possible that the common bands are due to more recent introgressions of *Bs1*-containing chromosomes from teosinte to maize. Since an increase in the copy number of a transposable element can only occur during transposition, the infrequency of transposition suggested by these data implies a low copy number for *Bs1*. The low transposition frequency may be an inherent property of *Bs1*, or it may be due to defects in *Bs1*'s structure. *Bs1* resembles a retrotransposon, but no studies have been performed that demonstrate its ability to function properly. *Bs1* differs significantly from *copia* and *Ty* by having no detectable RNA (necessary for transposition) in any of several tissues examined.

Another possible explanation for the low copy number of *Bs1* is that many *Bs1* insertions may cause lethality. This lethality might result from the direct effects of the insertion: inactivation of the target gene by inappropriate activation of a *Bs1*-based promoter. Alternatively, *Bs1* might be a site for "ectopic" recombination, as is found in *Saccharomyces cerevisiae*, where homologous recombinations between *Ty* elements at different sites lead to a variety of chromosomal abnormalities (Roeder and Fink 1980; Kupiec and Petes 1988).

A third possible reason for the low copy number is that *Bs1* may be under the constraint of a specific, copy control mechanism. A copy number control mechanism has been demonstrated for another maize transposable element, *Mutator*. The number of *Mutator* elements in the offspring of crosses involving active *Mutator* lines is roughly equal to the number in the active parent and not to the sum of the parental numbers, as would be expected without a copy number, compensating mechanism. A self-activated, negative regulator for *Mutator* copy number has been proposed (Alleman and Freeling 1986; Bennetzen 1987).

According to systematic evidence, *Tripsacum* is the genus most closely related to *Zea*. *Zea* can be divided into section *Luxuriantes* and section *Zea*. The former section, containing two perennial and one annual species, is thought to be the more primitive. The latter section, containing only one species that includes the Mexican annual teosintes, *Z. m. parviglumis* and *Z. m. mexicana*, is thought to be directly ancestral to maize (Doebley and Iltis 1980). The data in this report support this scenario. The *Bs1* probe hybridizes with *Tripsacum* DNA more strongly than with any of the other Andropogoneae, but not as well as with *Zea* DNA. Thus, *Zea* is probably more closely related to *Tripsacum* than to any of the other tested species. The presence of common bands in many of the *Z. m. mays*, *Z. M. parviglumis*, and *Z. m. mexicana* lines suggests that they are, indeed, more closely related to each other than to the teosintes of section *Luxuriantes*, which do not have the common bands. This supports the taxonomy of Doebley and Iltis (1980), while casting doubt on the suggestion that *Z. luxurians* or *Z. diploperennis* is directly ancestral to maize (Galinat 1988; Mangelsdorf 1986).

Acknowledgements. We wish to thank Drs. J. Doebley, R. Pohl, J. Wendel, C. Dewald, A. Hooker, and T. Zinnen, and the Lifaco Seed Co. for gifts of plant material or seed. We also thank Dr. B. Burr for the recombinant inbred lines and for computer analysis of the resulting data.

References

- Alleman M, Freeling M (1986) The *Mu* transposable elements of maize: evidence for transposition and copy number regulation during development. *Genetics* 112:107–119
- Bennetzen JL (1987) Covalent DNA modification and the regulation of *Mutator* element transposition in maize. *Mol Genet* 208:45–51
- Boeke JD, Garfinkel DJ, Styles CA, Fink GR (1985) *Ty* elements transpose through an RNA intermediate. *Cell* 40:285–294
- Burr B, Burr FA, Thompson KH, Albertson MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. *Genetics* 118:519–526
- Doebley JF, Iltis HH (1980) Taxonomy of *Zea* (Gramineae). I. A subgeneric classification with key to taxa. *Am J Bot* 98:982–983
- Doolittle RF, Feng DF, Johnson MS, McClure MA (1989) Origins and evolutionary relationships of retroviruses. *Q Rev Biol* 64:1–30
- Feinberg A, Vogelstein B (1982) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 132:6–13
- Galinat WC (1988) The origin of corn. In: Sprague GF, Dudley JW (eds) *Corn and Corn improvement*. American Society of Agronomy, Madison WI, pp 1–31
- Gould F, Shaw R (1968) *Grass systematics*, 2nd edn. Texas A&M University Press, College Station/TX
- Grandbastien MA, Spielmann A, Caboche M (1989) *Int1*, a mobile retrovirus-like transposable element of tobacco isolated by plant cell genetics. *Nature* 337:376–380

- Hitchcock AS (1935) Manual to the grasses of the United States. US Government Printing Office, Washington, D.C.
- Iltis HH, Doebley JF (1980) Taxonomy of *Zea mays* complex and a generic synopsis. *Am J Bot* 67:982-993
- Jin Y-K, Bennetzen JL (1989) The structure and coding properties of *Bs1*, a maize retrovirus-like transposon. *Proc Natl Acad Sci USA* 86:6235-6239
- Johns MA, Mottinger J, Freeling M (1985) A low copy number *copia*-like transposon in maize. *EMBO J* 4:1093-1102
- Johns MA, Babcock MS, Fuerstenberg SM, Fuerstenberg SI, Freeling M, Simpson RB (1989) An unusually compact retrotransposon in maize. *Plant Mol Biol* 12:633-642
- Kochetkov NK, Budovski EI (1972) Organic chemistry of nucleic acids. Plenum, London, pp 477-532
- Kupiec M, Petes TD (1988) Allelic and ectopic recombination between *Ty* elements in yeast. *Genetics* 119:549-559
- Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor/NY
- Mangelsdorf PC (1986) The origin of corn. *Sci Am* 255:80-86
- Montgomery E, Charlesworth B, Langley CH (1987) A test for the role of natural selection in the stabilization of copy number in a population of *Drosophila melanogaster*. *Genet Res* 49:31-41
- Mottinger JP, Johns MA, Freeling M (1984) Mutations of the *Adh1* gene in maize following infection with barley stripe mosaic virus. *Mol Gen Genet* 195:367-369
- Rhoades MM, Dempsey E (1982) The induction of mutable systems in plants with the high-loss mechanism. *Maize Genet Coop Newsl* 56:21-26
- Roeder GS, Fink GR (1980) DNA rearrangements associated with a transposable element in yeast. *Cell* 21:239-249
- Saghai-Marooif MA, Soiman KM, Jorgenson RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci USA* 81:8014-8018
- Southern EM (1975) Detection of specific sequences among DNA fragments separated by electrophoresis. *J Mol Biol* 98:503-517
- Stuber CW, Edwards MD (1986) Genotypic selection for improvement of quantitative traits using molecular marker loci. *Proc 41st Ann Corn Sorg Res Conf, Chicago IL*, pp 70-83
- Temin HM (1987) Evolution of retroviruses and other retrotranscripts. In: Bolognesi D (ed) Human retroviruses, cancer, and AIDS. UCLA Symp Mol Cell Biol, New Ser 71:1-28. Alan R. Liss, New York
- Varmus HE (1983) Retroviruses. In: Shapiro AJ (ed) Mobile genetic elements. Academic Press, New York, pp 441-503
- Voytas DF, Ausubel FM (1988) A *copia*-like transposable element family in *Arabidopsis thaliana*. *Nature* 336:242-244