Transposition Pattern of the Maize Element *Ac* from the *bz-m2(Ac)* Allele

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ABSTRACT

The pattern of transposition of *Ac* from the mutable allele *bz-m2(Ac)* has been investigated. Stable (bz-s) and finely spotted (bz-m(F)) exceptions were selected from coarsely spotted *bz-m2(Ac)* ears. The presence or absence of a transposed *Ac* (tr*Ac*) in the genome was determined and, when present, the location of the tr*Ac* was mapped relative to the flanking markers *sh* and *wx*. The salient general features of *Ac* transposition to sites linked to *bz* are that the receptor sites tend to be clustered on either side of the *bz* donor site and that transposition is bidirectional and nonpolar. Thus, the symmetrical clustering in the distribution of receptor sites adjacent to *bz* differs from the asymmetrical clustering reported in 1984 for the *P* locus by I. M. GREENBLATT. Though *Ac* tends to transpose preferentially to closely linked sites, an appreciable fraction of *Ac* transpositions from *bz-m2(Ac)* is to unlinked sites: 41% among bz-s derivatives and 59% among bz-m(F) derivatives. Many transposition events among the bz-m(F) selections result in kernels carrying a genetically noncorresponding embryo. These can be interpreted as twin sectors arising at one of the megagametophytic mitoses. The *bz* locus data fit the earlier (1962) model of I. M. GREENBLATT and R. A. BRINK in which transposition takes place from a replicated donor site to either an unreplicated or replicated receptor site.

OVER the last few years, plant transposable elements have become powerful gene isolation tools (FEDOROFF, FURTEK and NELSON 1984; DOONER *et al.* 1985; O'REILLY *et al.* 1985; MARTIN *et al.* 1985; PAZ ARES *et al.* 1986; CONE, BURR and BURR 1986; WIENAND *et al.* 1986; LECHELT, LAIRD and STARLINGER 1986; PETERSON and SCHWARTZ 1986; SCHMIDT, BURR and BURR 1987; THERES and STARLINGER 1987; MCLAUGHLIN and WALBOT 1987; MCCARTY *et al.* 1987). The increased use of transposons as tags to physically mark and isolate genes makes it imperative that, in order to optimize the efficiency of experiments that aim to recover a transposon at a desired target locus, we fully understand the pattern of transposition of the different elements at different donor loci.

The best characterized transposition behavior of any plant transposon is that of the *Ac* element present at the *P-vv* allele, conditioning variegated pericarp color in maize. Early on, VAN SCHAIK and BRINK (1959) showed that *Ac* at *P* (then called *Mp*, for Modulator of pericarp) transposed preferentially to closely linked sites. The subsequent extensive studies of GREENBLATT and BRINK (1962) and GREENBLATT (1984) have provided the basis for most of our current knowledge on the distribution of receptor sites for transposed *Ac* elements (tr*Ac*s) in the genome.

GREENBLATT mapped over 100 cases of transposition of *Ac* from the *P* locus. He found that in about 61% of cases, *Ac* was still linked to *P* and in the remaining 39%, *Ac* segregated independently of the *P* locus. He also found that *Ac* transposed preferentially to sites closely linked to *P*, that *Ac* could transpose both proximally and distally, but that, peculiarly, there was a 4-cM region immediately proximal to *P* where *Ac* did not appear to transpose. Based on these and other observations, GREENBLATT proposed a model of *Ac* transposition in which *Ac* transposes at the time of chromosome replication and inserts only into a region that is being replicated. The 4-cM gap can be explained if a replicon initiation site is located proximal to *P* so that the region has already completed replication at the time the *P* locus is replicated.

These observations are intriguing. The present investigation of the pattern of transposition of *Ac* from the *bronze* mutable allele *bz-m2(Ac)* was initiated several years ago in order to determine to what extent the *P* locus observations could be generalized to other loci. The advantages of the *bz* locus as a genetic system for this type of investigation are twofold: it conditions a seed phenotype, which facilitates the preselection of tr*Ac*s, and it is flanked by two easily scored endosperm markers, *sh* and *wx*, which expedites the subsequent mapping of the receptor sites. The issue of distribution of receptor sites for tr*Ac*s can be examined by analyzing derivatives that occur as single kernel events in ears carrying the *Ac* mutable allele *bz-m2(Ac)*. The information gained from such analysis on the pattern of transposition of *Ac* from its donor site in *bz-m2(Ac)* is the subject of this report.

[1] Current name: DNA Plant Technology Corporation.

MATERIALS AND METHODS

Description of *bz* alleles: All the alleles used in the present investigation were incorporated into the genetic background of the inbred W22. The aleurone phenotypes conditioned in the presence of all the complementary factors necessary for anthocyanin pigmentation are given in parentheses.

Bz-McC (purple): the normal progenitor allele of the *Ac* and *Ds* insertion mutations utilized in this study.

bz-m2(Ac) (bronze-purple variegation): an allele arising from insertion of a 4.6-kb *Ac* element in the second exon of *Bz-McC* (McCLINTOCK 1955; RALSTON, ENGLISH and DOONER 1988).

bz-m2(DI) (bronze in the absence of *Ac*; spotted, in its presence): the first derivative from *bz-m2(Ac)*, harboring a 3.3-kb *Ds* element as a consequence of an internal deletion from *Ac* (McCLINTOCK 1962; DOONER *et al.* 1986).

bz-R (bronze): the stable, reference allele at the locus; it is associated with a 340-bp deletion in the transcribed region (RHOADES 1952; RALSTON, ENGLISH and DOONER 1987).

Markers: The mutations *sh* (shrunken endosperm) and *wx* (waxy endosperm) were used as markers flanking the *bz* locus. They map, respectively, about 3 units distal and 25 units proximal to *bz* in *9S*. Chiasma interference in the region is very high. In one experiment involving 1072 individuals, its value was measured as one (DOONER 1986).

Selection and analysis of stable bronze and fine spotted derivatives from *bz-m2(Ac)*: These derivatives were selected as single kernel events from test-crosses of *sh bz-m2(Ac) Wx* homozygotes or *Sh bz-m2(Ac) Wx/sh bz wx* heterozygotes to *sh bz-R wx* pollen parents. An important consideration in the latter cross is that due to the high chiasma interference in the *sh wx* region ($I = 1$), practically all stable bronze types carrying the *Sh Wx* flanking markers of the *bz-m2(Ac)* allele will originate from an *Ac* transposition event rather than from a double crossing-over event. The derivatives were then crossed to a marked *Ds* tester stock, *sh bz-m2(DI) wx*, and the corresponding heterozygotes were test-crossed with *sh bz-R wx* to map the location of the tr*Ac* elements.

Molecular analysis: Restriction enzyme digestions, DNA isolation, and genomic blotting were carried out as described previously (DOONER *et al.* 1985).

RESULTS

Mutations from *bz-m2(Ac)*: As shown in Figure 1, *bz-m2(Ac)* can mutate from a normally coarse spotting type to either full purple, stable bronze or a fine spotting type (McCLINTOCK 1956). These exceptions arise from excisive transposition of the transposable element *Ac* from the *bz* locus, and, therefore, are highly likely to carry a tr*Ac*. When plant transposable elements excise, they usually do so imprecisely, so that the excision product retains part of the short direct repeat generated upon insertion (see review by DORING and STARLINGER 1986). Since the *Ac* element in *bz-m2(Ac)* is inserted in the second exon of *bz* (RALSTON, ENGLISH and DOONER 1988), it will often leave behind an impaired *bronze* gene upon excision. Therefore, the most common type of derivative is stable bronze (bz-s), followed by a fine mutable type (bz-m:F), indicative of an increase in *Ac* dosage, and a purple type (Bz), corresponding to restorations of *bz* locus function. In one experiment involving 5650 *bz-m2* gametes, the aforementioned exceptions occurred in the following percentages: bz-s, 1.7%, bz-m(F), 1.5%, and Bz, 0.4%. The two types of derivatives analyzed in this study are bz-s and bz-m(F).

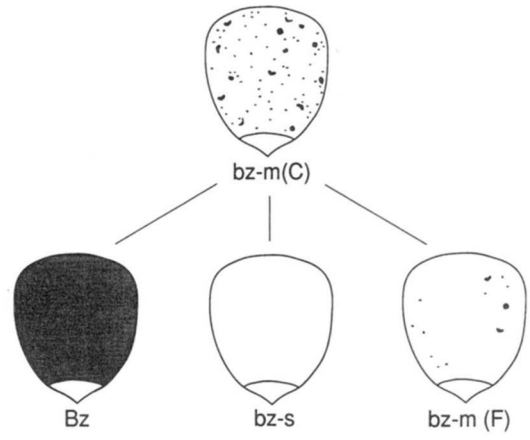

FIGURE 1.—Phenotypes of *bz-m2(Ac)* and its derivatives. bz-m(C), coarsely spotted; Bz, purple; bz-s, stable bronze; bz-m(F), finely spotted.

Analysis of stable bronze derivatives: New stable *bz* alleles originate from excision of *Ac* from *bz-m2(Ac)*. They are collectively designated *bz-s* and each new allele is identified by the four digits of the pedigree number under which it was first grown (*e.g.*, *bz-s:2097*). The presence or absence of *Ac* in each *bz-s* stock can be ascertained by crossing to a *Ds* reporter stock, such as *bz-m2(DI)*, and scoring for the presence or absence of variegation.

The location of tr*Ac* is then determined from test-crosses of *Sh bz-s Wx* (tr*Ac*)/*sh bz-m2(DI) wx* heterozygotes to *sh bz-R wx*, by selecting spotted seed and classifying flanking markers. There are various distinct outcomes depending on whether *Ac* has transposed between *sh* and *bz*, between *bz* and *wx*, distal to *sh*, proximal to *wx*, or to an unlinked site (Figure 2). If *Ac* is located in the *sh-bz* interval (Figure 2A), crossing over between *Ac* and *bz-m2(DI)* will produce plump waxy (Sh wx) spotted seeds. This class should represent less than 1–2% of the total kernel population, or less than half the size of the *sh-bz* interval (only half of the crossovers can be detected). If *Ac* is located in the *bz-wx* interval (Figure 2B), crossing over between *bz-m2(DI)* and *Ac* will produce shrunken non-waxy (sh Wx) spotted seed. Again, this class should represent less than half the recombination percentage between *bz* and *wx*. If *Ac* is located distal to *sh*, recombination events between *Ac* and *sh* will give rise to shrunken waxy (sh wx) spotted seeds and those between *sh* and *bz* will give rise to plump waxy (Sh wx) spotted seeds (Figure 2C). The size of the former class will vary depending on the location of *Ac*, but the latter class should represent about 1–2% of the total

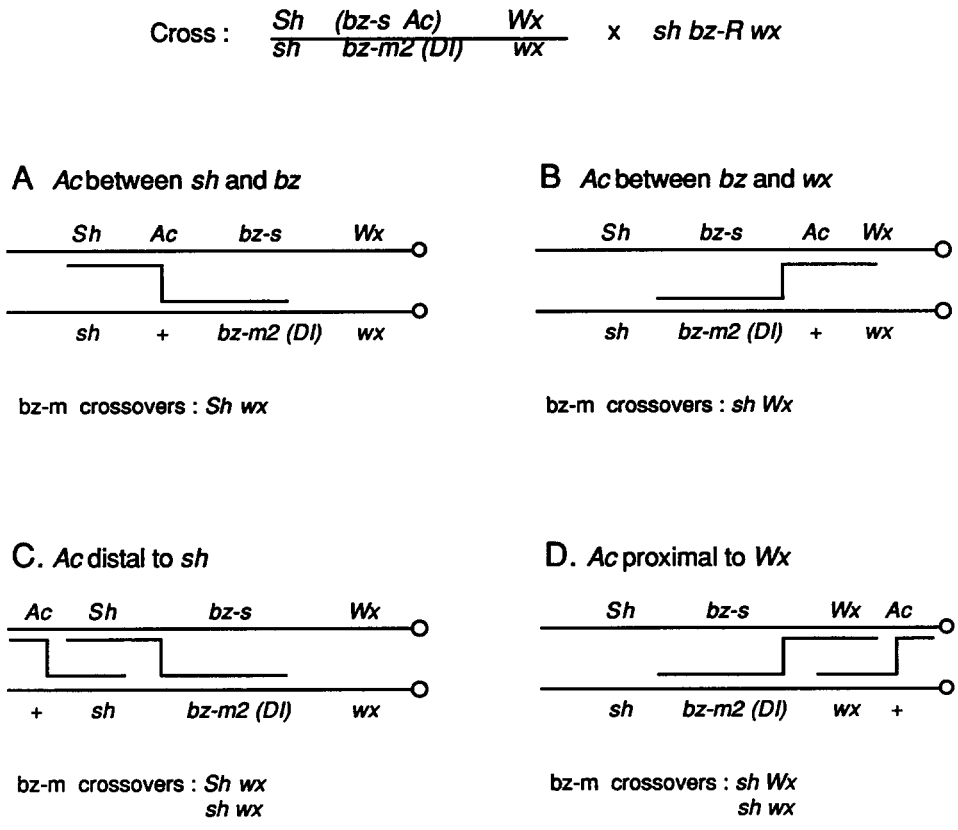

FIGURE 2.—Scheme for mapping transposed *Ac* among *bz-s* derivatives from *bz-m2(Ac)*. Spotted (bz-m) seeds are selected from the crosses indicated above and scored for outside markers. Different crossover types are recovered depending on the location of the tr*Ac*.

TABLE 1

Summary of the analysis of bz-s types from *bz-m2(Ac)*

No. analyzed	*Ac* present			*Ac* absent
	Total	Linked	Unlinked	
116	49 (0.42)	29	20	67 (0.58)

(half the size of the *sh-bz* interval). If *Ac* is located proximal to *wx*, there will also be two types of spotted kernel recombinants depending on the position of the exchange: recombination between *bz* and *wx* will generate sh Wx seeds, whereas recombination between *wx* and *Ac* will generate sh wx seeds. The former class should constitute about 10% of the total or half the recombination percentage between *bz* and *wx*, whereas the latter class will be of variable size. Finally, if *Ac* segregates independently of *bz*, one quarter of the kernels will be spotted and among them, the majority will be sh wx.

Table 1 shows the summary of the analysis of *bz-s* types from *bz-m2(Ac)*. Of 116 cases examined, *Ac* was present in 49 (42%) and absent in 67 (58%). Among those *bz-s* derivatives having a tr*Ac*, 29 (59%) carried a linked *Ac* and 20 (41%) carried an unlinked *Ac*. It is evident from the number of *bz-s* derivatives carrying an unlinked *Ac* that loss of a tr*Ac* by meiotic segregation can account for only a fraction of the *bz-s* derivatives that lack *Ac*. Other possible mechanisms to explain their origin are transposition to the sister chromatid, inactivation of the *Ac* element or excision of *Ac* without reinsertion.

Loss of *Ac* activity at the *wx* locus has been correlated with methylation (modification) of internal sites in *Ac* (SCHWARTZ and DENNIS 1986; CHOMET, WESSLER and DELLAPORTA 1987). In order to investigate the possibility that inactivation of the *Ac* element at the *bz* locus might contribute to the class of *bz-s* derivatives lacking a genetically active *Ac* element, 24 such derivatives were subjected to Southern blot analysis. Figure 3A shows a representative blot in which *Bgl*II digests of DNA from *bz-m2(Ac)* and several *bz-s* derivatives were hybridized with a *bz*-specific probe. The individuals in lanes 11 and 12 are *bz-m2(Ac)/bz-R* heterozygotes and exhibit the expected 11.2-kb *bz-m2(Ac)* band and 6.5-kb *bz-R* band (DOONER *et al.* 1985; RALSTON, ENGLISH and DOONER 1987). The individuals in lanes 1–5 and 7–10 are *bz-s/bz-m2(DI)* heterozygotes. All exhibit an identical pattern, the expected 9.7-kb *bz-m2(DI)* band and a 6.5-kb band that corresponds to the *bz* empty site, indicating that these derivatives arise by excision of *Ac*. A similar empty site was detected in all other *Ac* minus, *bz-s* derivatives examined.

Further evidence against *Ac* inactivation at *bz* comes from *Pvu*II digests. *Pvu*II is a methylation sensitive enzyme which produces a characteristic 2.5-kb *Ac*-homologous fragment in stocks carrying an active *Ac*

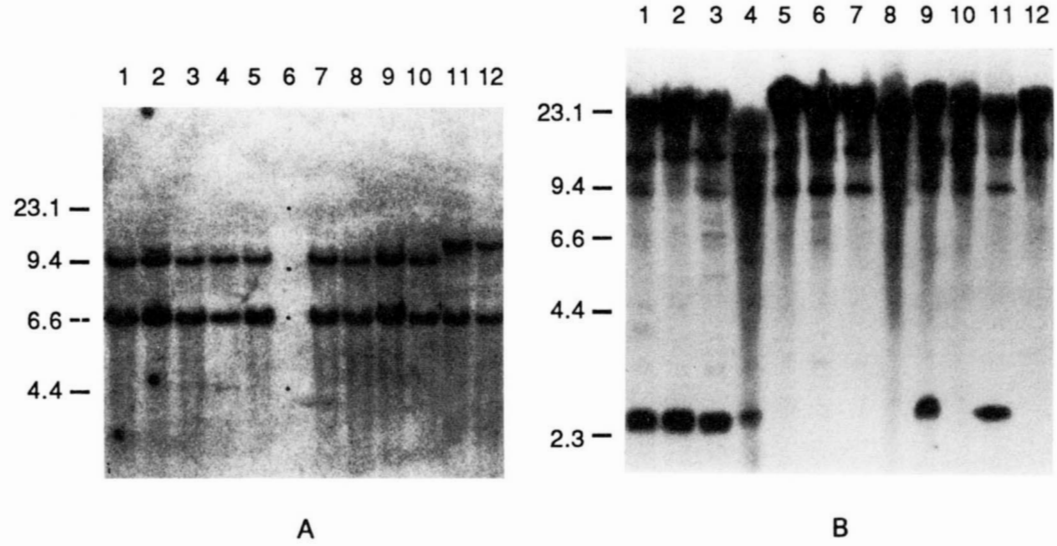

FIGURE 3.—Southern blot analysis of *bz-s* derivatives. (A) *Bgl*II digests hybridized to the *bz*-specific probe pAGS528 (DOONER *et al.* 1985). Lanes 1–5 and 7–10: *bz-s/bz-m2(DI)* heterozygotes; lanes 11 and 12: *bz-m2(Ac)/bz-R* heterozygotes; lane 6: molecular weight markers. (B) *Pvu*II digests hybridized to pAGS501, the internal 1.6-kb *Hin*dIII fragment from *Ac* (DOONER *et al.* 1985). Lane 1: *bz-m2(Ac)/bz-R*; lanes 2–4, 9 and 11: *bz-s/bz-R* heterozygotes, *Ac* present; lanes 5–8, 10 and 12: *bz-s/bz-R* heterozygotes, *Ac* absent.

element or larger fragments in stocks carrying an inactive *Ac* element at the *wx* locus (SCHWARTZ and DENNIS 1986; CHOMET, WESSLER and DELLAPORTA 1987). Figure 3B shows a Southern blot analysis of several *bz-s* derivatives that either have or lack *Ac*. Hybridization of *Pvu*II digests with an *Ac*-homologous probe reveals the presence of a 2.5-kb band in stocks carrying an active *Ac* (lanes 1–4, 9 and 11) and its absence in stocks lacking an active *Ac* (lanes 5–8, 10 and 12). None of the 24 *Ac* minus, *bz-s* derivatives tested produced a 2.5-kb band or any other distinct band that hybridized to *Ac*. We conclude that the majority of this class of derivatives originates by *Ac* excision without reinsertion, by sister chromatid transposition or by transposition to locations in the genome where *Ac* becomes inactivated by methylation. Evidence that such *Ac* elements may exist was obtained by DOONER *et al.* (1985), who isolated from a *bz-m2(Ac)* stock with a single active *Ac* at *bz* an element similar to *Ac* in size and restriction map that had inserted in highly repetitive DNA and that was probably genetically inert.

For those transposed *Ac* elements linked to the *bz* donor site, the estimates of genetic distance between *Ac* and *bz* are based on testcross progenies of at least 1000 kernels each. Table 2 presents the pertinent mapping data. An examination of the data reveals the following salient points.

1. In most cases there is a majority recombinant class, so that the proximal-distal orientation of tr*Ac* relative to *bz* can be readily established. In the few instances where there is no obvious majority recombinant class (*e.g.*, *bz-s:6087*), the few individuals carrying recombinant arrangements of outside markers were progeny tested further to elucidate the *bz-Ac* linkage relationship. The *Sh wx* and *sh Wx* spotted exceptions (*bz-m2(DI)/bz-R* Ac/+) were backcrossed to the *sh bz-R wx* tester. True crossovers, in which tr*Ac* is linked to the *Ds* reporter allele, will segregate approximately 1 bz-m: 1 bz; secondary transpositions of tr*Ac* to unlinked sites will segregate 1 bz-m: 3 bz. In all the cases examined, the *Sh wx* exceptions segregated 1:1, whereas the *sh Wx* exceptions segregated 1:3, indicating that tr*Ac* was closely distal to *bz*. The basis for the absence of a distinct majority recombinant class among *bz-s* derivatives in which tr*Ac* is closer than 0.2 cM on the distal side of *bz* is the inequality in genetic lengths of the two flanking intervals, *sh-bz* and *bz-wx*, which leads to a fortuitous balance between the number of true crossovers (Sh wx) and the number of secondary transpositions to unlinked sites that are accompanied by an exchange in the larger *bz-wx* interval (sh Wx).

2. In two derivatives, *bz-s:2114* and *bz-s:7055*, *Ac* appears to reside at *bz*. Southern analysis of these derivatives indicates that they carry deletions immediately adjacent to the *Ac* insertion site (DOONER 1985 and our unpublished results). This inference has been confirmed by cloning and sequencing the *bz-s:2114* allele, which arose by deletion of 789 bp proximal to *Ac* (DOONER, ENGLISH and RALSTON 1988). Since in these derivatives *Ac* still resides at the *bz* locus, the alleles have been designated *bz-s:2114(Ac)* and *bz-s:7055(Ac)*.

3. Among *bz-s* derivatives carrying a transposed *Ac* element within the *sh-wx* interval, the parentally marked *sh wx* spotted class represents principally secondary transpositions of *Ac* to unlinked sites. This has

TABLE 2

Placement of linked tr*Ac*s among *bz-s* selections from *bz-m2(Ac)*[a]

Allele	Flanking markers among bz-m kernels				No. of kernels	Map order	*bz-Ac distance (cM)*
	Sh Wx	*sh wx*	*Sh wx*	*sh Wx*			
6065	0	200[b]	28	7	2536	*Ac sh bz wx*	17.5
7050	0	104[b]	16	1	1538	*Ac sh bz wx*	15.1
6088	2	133[b]	17	6	2233	*Ac sh bz wx*	13.6
6077	1	51[b]	31	8	2283	*Ac sh bz wx*	6.7
7053	0	38[b]	26	2	2559	*Ac sh bz wx*	4.5
6089	1	14	25	3	2694	*Ac sh bz wx*	2.4
7054	1	1	17	1	1813	*sh Ac bz wx*	1.9
6083	4	10	53	4	9458	*sh Ac bz wx*	1.1
7051	6	5	10	1	2450	*sh Ac bz wx*	0.8
6070	4	1	7	0	2521	*sh Ac bz wx*	0.6
2103	2	4	7	2	2913	*sh Ac bz wx*	0.5
6067	14	13	13	8	8449	*sh Ac bz wx*	0.3
6058	1	30	8	5	8210	*sh Ac bz wx*	0.2
6087	10	15	5	8	8237	*sh Ac bz wx*	0.1
7077[c]	0	1	2	1	1574	*sh Ac bz wx*	0.1
2114	0	20	1	1	7898	*Ac* at *bz*	0.0
7055	0	3	0	0	1233	*Ac* at *bz*	0.0
2094	0	10	0	7	20277	*sh bz Ac wx*	0.05
2116	0	3	0	6	3451	*sh bz Ac wx*	0.3
7056	2	1	0	3	1711	*sh bz Ac wx*	0.4
3137	0	8	0	18	2488	*sh bz Ac wx*	1.4
2106	1	8	0	51	3241	*sh bz Ac wx*	3.1
2097	0	8	0	28	1641	*sh bz Ac wx*	3.3
3136	0	16	0	31	1698	*sh bz Ac wx*	3.7
6056	1	8	0	86	1687	*sh bz Ac wx*	10
6066	0	2	0	91	1533	*sh bz Ac wx*	12
6085	0	46[d]	0	228	3052	*sh bz wx Ac*	17.5
2107	0	22[d]	0	130	1401	*sh bz wx Ac*	21.2
6084	0	50[d]	0	173	1857	*sh bz wx Ac*	23.5
6064	0	92[d]	0	124	1328	*sh bz wx Ac*	32.0

[a] From crosses of the type *Sh bz-s Wx* [*Ac*]/ *sh bz-m2(DI) wx* × *sh bz-R wx*.
[b] Includes *Ac-sh* crossovers and transpositions to unlinked sites.
[c] bz-s derivative from a bz-m(F) selection.
[d] Includes *wx-Ac* crossovers and transpositions to unlinked sites.

been demonstrated by testcrossing a group of these exceptions to *bz-R* and verifying that they segregate 1 bz-m: 3bz (DOONER, RALSTON and ENGLISH 1988). Therefore, the frequency of this class provides an estimate of the frequency of secondary transposition of *Ac* from its new location in the genome to unlinked sites. For most tr*Ac*s, this frequency is less than 1 in 100, but can range from less than 1×10^{-3} for the tr*Ac* element in the *bz-s:2094* derivative, which carries in addition a 2-kb single-ended, fractured *Ac* element at *bz* (E. J. RALSTON, J. ENGLISH AND H. K. DOONER, in preparation), to a high of 1.8% for the tr*Ac* in the *bz-s:3136* derivative, the mean being 5×10^{-3}. (Frequencies have been adjusted by a factor of 2, since transposition to unlinked sites can be detected in only half the gametes.) The variability in secondary transposition frequencies among tr*Ac* elements derived from the *P-vv* allele has also been reported by VAN SCHAIK and BRINK (1959).

4. Among *bz-s* derivatives carrying a tr*Ac* outside the *sh-wx* interval, the sh wx spotted class (marked with superscripts in Table 2) includes both secondary transpositions and crossovers between *Ac* and either flanking marker, the ratio of their respective contributions being inversely related to the distance separating *Ac* from the flanking marker. Based on the preceding paragraph, one can expect that the contribution of secondary transpositions to the sh wx class will be generally low, 0.5% on average. Since no attempt was made to separate transpositions from crossovers in most cases, the distance between *bz* and *Ac* among these derivatives was estimated by adding the percentage of the sh wx class, corrected by 0.5% to account for secondary transpositions, to that of either the Sh wx or the sh Wx recombinant class.

5. Sh Wx spotted exceptions constitute, by far, the smallest flanking marker class in Table 2. This is to be expected since, formally, they represent double crossovers in the *sh wx* region, where interference is high. However, in the group of seven *bz-s* derivatives with an *Ac* element within 1 cM distally from *bz*, Sh Wx is not the smallest class. In fact, the frequency of the Sh bz-m Wx class is higher in these *bz-s* derivatives (37/34354 or 0.11%) than in the eight *bz-s* derivatives carrying *Ac* farther than 1 cM distally from *bz* (9/25564 or 0.04%). This result is the *opposite* of what one would expect if Sh Wx spotted types arose by double crossing over. However, it is in agreement with our earlier observations on intragenic recombination between transposable element insertion mutations (DOONER and KERMICLE 1986; DOONER 1986). We found that recombination between chromosomes bearing closely linked insertions produces predominantly parentally marked exceptions. Thus, the occurrence of Sh bz-m Wx types among *bz-s* derivatives carrying a distal *Ac* element closely linked to *bz* can be explained by recombination events between tr*Ac* and *Ds* that are resolved without flanking marker exchange, *i.e.*, by conversion of the *bz-m2(DI)* reporter allele. The reciprocal conversion event of the tr*Ac* site may also occur. It would give rise to a sh bz-m wx kernel type, which would only be distinguished from a phenotypically identical secondary transposition by progeny testing. No attempt was made in this study to sort out the relative contributions of these two sources of sh bz-m wx kernels.

The distribution of linked tr*Ac*s in *9S* is shown graphically in Figure 4. An examination of this figure leads to the following conclusions. First, as in the *P* locus data, there is an obvious clustering of receptor sites closely linked to the *bz* donor site. There are two 0 values: these are real and correspond to deletions adjacent to the *Ac* insertion site in *bz*. Second, *Ac* appears to transpose proximally and distally with about equal frequency. Third, in contrast to the *P*

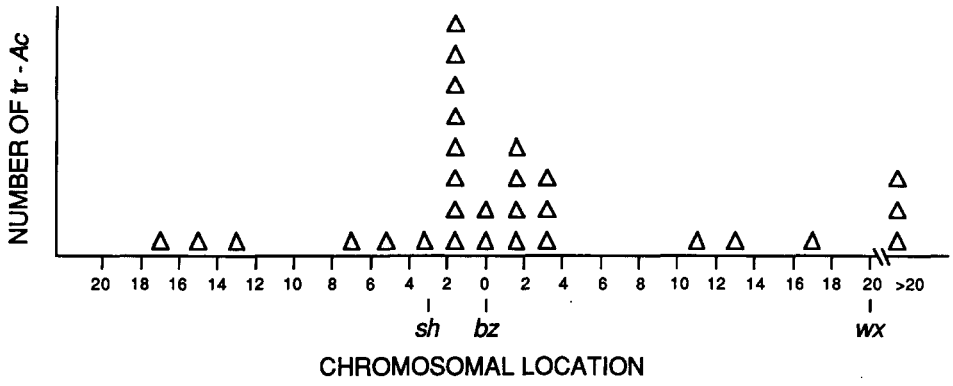

FIGURE 4.—Distribution of linked tr*Ac*s among *bz-s* selections from *bz-m2(Ac)*. The position of the donor *bz* locus is indicated as 0. Locations distal to *bz* are to the left and those proximal to *bz* are to the right of *bz*.

locus data, there is no region devoid of tr*Ac*s either immediately to the left or right of *bz*.

Analysis of finely spotted derivatives: A well known property of *Ac* is its "negative dosage effect," *i.e.*, as *Ac* dosage is increased, there is a delay in timing and a reduction in frequency of transposition of *Ac* (McCLINTOCK 1951; BRINK and NILAN 1952). The spots in a spotted kernel become fewer and finer, so that kernels with a fine spotting phenotype in ears carrying *bz-m2(Ac)* represent instances where *Ac* dosage has increased as a consequence of an *Ac* transposition event. Such derivatives are collectively referred to as bz-m(F), a convenient phenotypic designation. In *bz-m2(Ac)* ears they constitute a clearly discrete phenotypic class, easily distinguishable from the majority of coarsely spotted kernels.

The location of tr*Ac*s among bz-m(F) derivatives was mapped in a similar fashion to the location of tr*Ac*s among *bz-s* derivatives. In the following discussion, (tr*Ac*) stands for a linked tr*Ac* of undetermined location in *9S*. Testcrosses of heterozygotes *Sh bz-m2(Ac) Wx* (tr*Ac*)/*sh bz-m2(DI) wx* produce a majority of either finely spotted or bronze seeds plus a few exceptional coarsely spotted seeds that carry a single *Ac* element. The exceptions, designated bz-m(C), were selected and classified for outside markers. As shown in Figure 5, various outcomes are possible, depending on where *Ac* is located. However, because the products of reciprocal recombination, *bz-m2(Ac)* and *bz-m2(DI)* (tr*Ac*), are phenotypically equivalent, the flanking marker classification of bz-m(C) exceptions is not a sufficient criterion to determine the position of the exchange (compare Figure 5, A and B). Southern blot analysis must be used to determine the *bz* locus constitution of bz-m(C) crossover exceptions in those cases where the tr*Ac* lies within approximately 3 cM of *bz*. For example, if tr*Ac* is located in the *sh-bz* interval (Figure 5A), recombination between tr*Ac* and *bz-m2(DI)* will produce Sh wx and sh Wx coarsely spotted seeds in about equal numbers. Southern blot analysis would establish that the former carry *bz-m2(DI)* and the latter carry *bz-m2(Ac)* at the *bz* locus. These exceptions should represent less than about 3% of the total, the percent recombination between *sh* and *bz*. *Ac* locations farther than about 3 cM from *bz* can be distinguished on the basis of genetic data alone, following a similar logic to that applied to the mapping of tr*Ac*s among *bz-s* derivatives.

The summary of the analysis of 68 bz-m(F) types from *bz-m2(Ac)* is presented in Table 3. Unlike the situation with the *bz-s* types, there are two classes of bz-m(F) derivatives with respect to transmission of the selected phenotype to the next generation: those with a concordant embryo and endosperm, in which the embryo carries a tr*Ac* besides the one present at *bz*, as expected from the selected finely spotted phenotype, and those with a nonconcordant embryo and endosperm, in which the genotype of the embryo is different from the expected *bz-m2(Ac)* + tr*Ac*.

Concordant bz-m(F) derivatives can arise from *Ac* transposition either at meiosis or at a postmeiotic division occurring early enough to lead to the recovery of a tr*Ac* in both the embryo and the endosperm. Of 48 tr*Ac*s in such derivatives, 20 (41%) are linked to *bz*, whereas 28 (59%) are unlinked. Nonconcordant bz-m(F) derivatives arise post-meiotically, during development of the female gametophyte. Of 23 such cases examined, 11 simply carried *bz-m2(Ac)* and 12 carried a *bz-s* allele. Among the *bz-s* types, *Ac* was present in 8 and absent in 4. Nonconcordant bz-m(F) derivatives carrying a *bz-s* allele in the embryo can be considered to represent twin sectors originating at the megagametophytic mitosis that gives rise to the egg and to its sister polar nucleus (COOPER 1937; SIMCOX, SHADLEY and WEBER 1987). Their possible origins will be discussed later.

The data for the placement of linked tr*Ac*s among the bz-m(F) selections are presented in Table 4. For all derivatives where *Ac* mapped within 3 cM from *bz*, Southern blot analysis of at least three bz-m(C) crossovers allowed the unambiguous placement of *Ac* relative to *bz*. For example, among the bz-m(C) offspring from derivative 7074, the 3 *Sh wx* crossovers examined carried *bz-m2(DI)* and the single *sh Wx* crossover carried *bz-m2(Ac)*, whereas among the bz-m(C) offspring from derivative 7067, the converse was true:

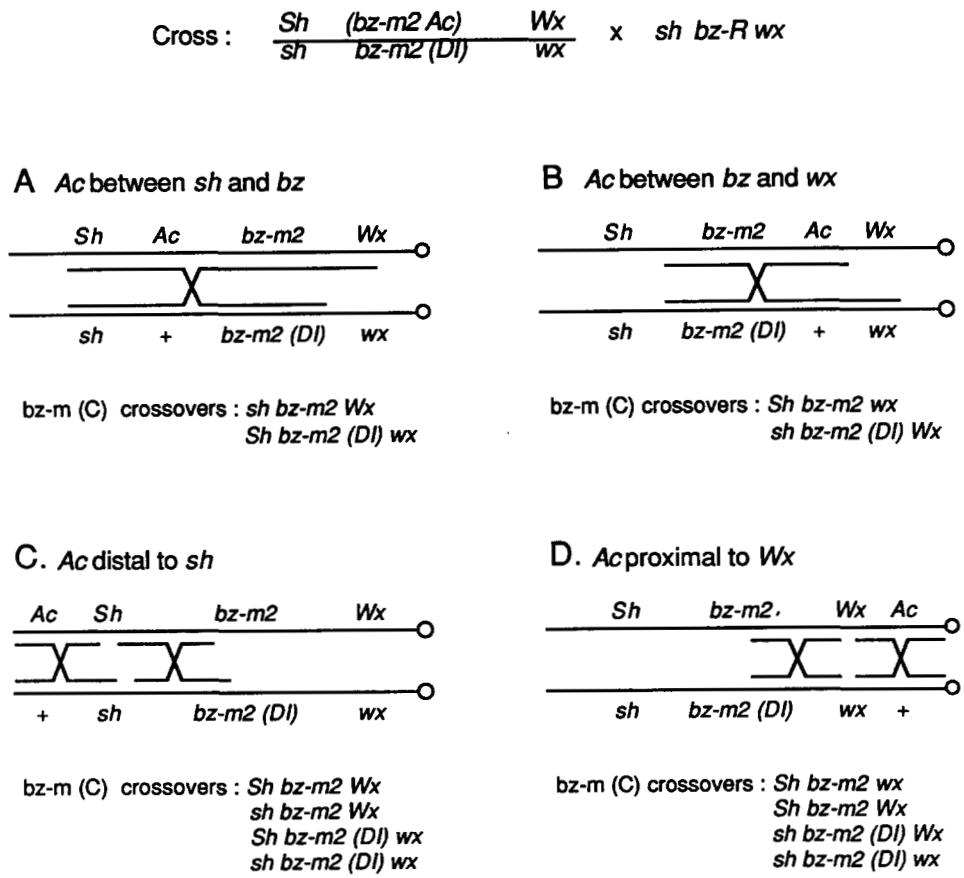

FIGURE 5.—Scheme for mapping transposed *Acs* among bz-m(F) derivatives from *bz-m2(Ac)*. Coarsely spotted (bz-m(C)) seeds are selected from the crosses indicated above and scored for outside markers. Different crossover types are recovered depending on the location of the tr*Ac*. Because the phenotypes of *bz-m2(Ac)* and *bz-m2(DI)* + *Ac* are equivalent, Southern blot analysis is used to determine the *bz* locus constitution of the bz-m(C) crossover products.

TABLE 3

Summary of the analysis of bz-m(F) types from *bz-m2(Ac)*

	Concordant embryo/endosperm (meiotic or postmeiotic)			Nonconcordant embryo/endosperm (postmeiotic)			
	bz-m2(Ac) + *Ac*			bz-s[a]			
No. analyzed	Total	Linked	Unlinked	Total	+*Ac*	−*Ac*	*bz-m2(Ac)*
68	45[b]	20	28	23	8	4	11

[a] Twin sectors.
[b] Three had 2 tr-*Acs*.

the *Sh wx* crossovers analyzed carried *bz-m2(Ac)* and the *sh Wx* crossovers carried *bz-m2(DI)*. As with the *bz-s* derivatives, a high frequency of parentally marked *sh wx* and *Sh Wx* exceptions is indicative of *Ac* transposition to sites outside the *sh-wx* marked interval. In those cases, the frequency of *Sh wx* and *sh Wx* recombinant classes was invariably close to either the *sh-bz* or *bz-wx* recombination values. For example, in derivative 6113, the frequency of parentally marked bz-m(C) kernels is high (19.3%) and the frequency of nonparentally marked bz-m(C) kernels is 3.3%, close to the actual recombination percentage between *sh* and *bz*. Thus, the genetic data suggests that *Ac* is distal to *sh*. Southern blot analysis of recombinant bz-m(C) individuals confirmed this placement.

In one case, 7081, the criteria described above were insufficient to allow an accurate placement of tr*Ac* relative to *sh*. The frequency of the nonparental classes was 2.2%, close to the *sh-bz* recombination percentage, and the frequency of parental classes was low (1.8%). Southern blot analysis of selected bz-m(C) crossovers placed *Ac* distal to *bz*. To locate tr*Ac* relative to *sh*, the testcross progeny were screened for the presence of *sh Wx* marked bz-m(F) kernels, which can only arise from an exchange between *sh* and a tr*Ac* located in the *sh-bz* interval. This class was, in fact, found (5/1498), thus allowing the placement of *Ac* proximal to *sh*.

As with the *bz-s* derivatives, secondary transpositions of either the *Ac* element at *bz* or the tr*Ac* will affect the estimates of genetic distance between *bz* and tr*Acs* lying outside the *sh-wx* marked region. Cosegregation of either newly transposed *Ac* with the *sh bz-m2(DI) wx* chromosome will result in *sh wx* parentally marked bz-m(C) offspring. Conversely, excision and loss of the primary tr*Ac* will result in *Sh Wx* parentally marked bz-m(C) offspring. However, since the percentage of parentally marked classes among bz-m(F) derivatives carrying a tr*Ac* within the interval delimited by the flanking markers *sh* and *wx* is low (somewhat variable, but around 1%), the frequency of secondary transpositions to unlinked sites can be assumed to be generally low. Therefore, estimates of genetic

TABLE 4

Placement of linked tr*Ac*s among bz-m(F) selections from *bz-m2(Ac)*[a]

Allele	Flanking markers among bz-m(C) kernels				No. of kernels	Map order	*bz-Ac* distance (cM)
	Sh Wx	*sh wx*	*Sh wx*	*sh Wx*			
6113[b]	103	76	17	14	927	*Ac sh bz wx*	18.3
6115	90	79	26	18	1151	*Ac sh bz wx*	17.5
7069	182	166	51	34	2424	*Ac sh bz wx*	16.9
7070	25	15	31	30	1966	*Ac sh bz wx*	4.1
7081	10	10	23	10	1498	*sh Ac bz wx*	2.2
7074	4	1	4	1	1093	*sh Ac bz wx*	0.5
7082	1	3	3	0	860	*sh Ac bz wx*	0.3
6114	7	3	2	1	1279	*sh bz Ac wx*	0.2
7065	4	10	4	7	1855	*sh bz Ac wx*	0.6
7071	10	13	6	6	1274	*sh bz Ac wx*	1.0
7072	2	4	9	6	1237	*sh bz Ac wx*	1.2
7067	3	7	6	10	1377	*sh bz Ac wx*	1.2
7068	13	9	7	3	796	*sh bz Ac wx*	1.3
7080	37	15	22	33	2673	*sh bz Ac wx*	2.1
6117	28	12	42	44	1820	*sh bz Ac wx*	4.7
7066[b]	0	1	31	17	411	*sh bz Ac wx*	12
7052	11	15	155	134	1550	*sh bz Ac wx*	17.6
6116[b]	27	30	63	65	664	*sh bz wx Ac*	26.8
7084	40	28	140	109	1103	*sh bz wx Ac*	27.7
6110[c]	41	0	104	0	896	*sh bz wx Ac*	30.8

[a] From crosses of the type *Sh bz-m2 Wx* [*Ac*]/ *sh bz-m2(DI) wx* × *sh bz-R wx*.

[b] An unlinked *Ac* was also present.

[c] From crosses of the type *Sh bz-m2 Wx* [*Ac*]/ *sh bz-R wx* × sh bz-*R wx*.

distance between *bz* and tr*Ac*s lying outside the *sh wx* region were obtained by adding the percentages of all classes of bz-m(C) exceptions and subtracting 1% to correct for secondary transpositions. This numerical manipulation may affect slightly the estimate of transposition distance but not the placement of *Ac* relative to the other *9S* markers.

A plot of the distribution of tr*Ac*s among bz-m(F) derivatives is presented in Figure 6. Though only 20 tr*Ac*s have been mapped, the following observations can be made. As with the *bz-s* derivatives, the tr*Ac*s are recovered in about equal numbers proximally and distally to *bz* and there is no obvious region devoid of tr*Ac*s either immediately to the left or right of *bz*. However, the clustering of tr*Ac*s around the donor site seen with *bz-s* derivatives is less pronounced.

Among the nonconcordant bz-m(F) derivatives which carried a *bz-s* allele and *Ac*, two appear by genomic Southern analysis to have arisen by deletion of at least part of the *bz* locus. One of them, *bz-s:7079*, may be an adjacent deletion, similar to *bz-s:2114(Ac)* and *bz-s:7055(Ac)*. The other one, *bz-s:7089*, is a *sh bz* double mutant. Both of them are being subjected to further molecular analysis to elucidate their origin.

DISCUSSION

The pattern of transposition of the *Ac* element present in the *bz-m2(Ac)* allele in the short arm of chromosome *9* (*9S*) has been examined in this report. There are similarities and differences with the pattern of transposition of the *Ac* element present in the *P-vv* allele in *1S*. As in *P-vv* (VAN SCHAIK and BRINK 1959; GREENBLATT 1984), there is a pronounced clustering of *Ac* receptor sites closely linked to the donor site. The preference shown by *Ac* for very closely linked sites may be even stronger for *bz* than for *P*. In contrast to *P-vv* (GREENBLATT 1984), however, the distribution of receptor sites adjacent to the donor site is symmetric. There is a roughly equal distribution of tr*Ac*s on either side of the *bz* donor site and, more importantly, there is no region devoid of receptor sites either immediately proximal or distal to *bz*.

If the pattern of transposition of *Ac* adjacent to *bz* were a reflection of a chromosome replication pattern, as GREENBLATT (1984) has suggested for *P*, the replicon initiation site would have to be either within *Ac* or very close to the *Ac* insertion site. According to GREENBLATT'S most recent replicon model (1984), *Ac* transposes during replication and reinserts only at sites that are as yet unreplicated. Though the symmetrical distribution of receptor sites immediately adjacent to *bz* neither supports nor disproves this model, the data presented here can best be explained by the simpler model of *Ac* transposition initially proposed by GREENBLATT and BRINK (1962) according to which *Ac* transposes during chromosome replication from a replicated site to either a replicated or an unreplicated site. That is, no restrictions were placed on the nature of the receptor site, which in GREENBLATT'S later model (1984) had to be a region that was actively replicating. The transposition of *Ac* to unreplicated sites, a feature of both models, has been demonstrated physically by CHEN, GREENBLATT and DELLAPORTA (1987). In the following discussion, various aspects of the *bz* locus data will be examined in light of the GREENBLATT and BRINK (1962) model.

The origin of the nonconcordant bz-m(F) derivatives carrying *bz-s* plus a tr*Ac* (Table 3) can be envisioned as shown in Figure 7. As in the GREENBLATT and BRINK (1962) model, *Ac* transposes after replication and into an unreplicated site. The consequence of such a transposition event will be that one sister chromatid carries *bz-s* plus a tr*Ac* and the other one carries *bz-m2(Ac)* plus a tr*Ac*. As drawn in the figure, the unreplicated receptor site lies in the same chromosome, but it could lie in a different chromosome, as well. If the egg receives the *bz-s* [tr*Ac*] chromatid and its sib polar nucleus receives the *bz-m2(Ac)* [tr*Ac*] sister chromatid, the kernel resulting from double fertilization will have a bz-m(F) endosperm and a bz-s [tr*Ac*] embryo.

The origin of the nonconcordant bz-m(F) derivatives where the embryo lacks *Ac*, a class of twin sector also seen by GREENBLATT and BRINK, can be explained

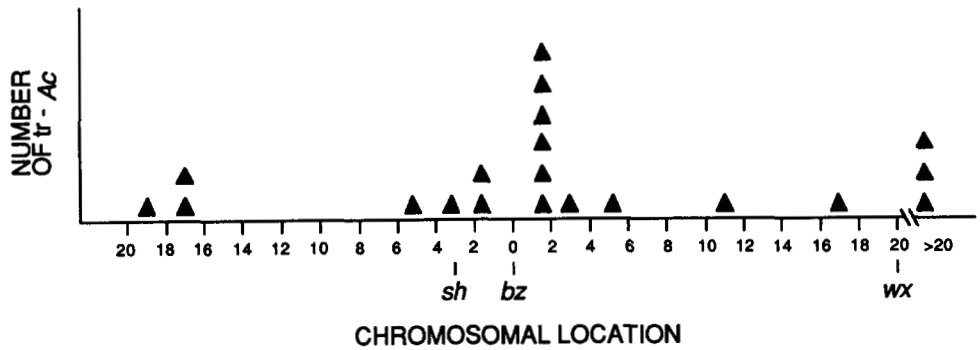

FIGURE 6.—Distribution of linked tr*Ac*s among bz-m(F) selections from *bz-m2(Ac)*. The position of the donor *bz* locus is indicated as 0. Locations distal to *bz* are to the left and those proximal to *bz* are to the right of *bz*.

according to their 1962 model as originating from transposition of *Ac* after replication and into an already replicated site (Figure 8). The consequence of this event will be sister chromatids carrying *bz-s* without *Ac* and *bz-m2(Ac)* with a tr*Ac*, respectively. In the figure, the replicated receptor site lies in the same replicon, but could also lie in a different replicon of the same chromosome or in a different chromosome. Again, if at the corresponding mitosis the egg receives *bz-s* and its sib polar nucleus receives *bz-m2(Ac)* plus a tr*Ac*, the kernel resulting from double fertilization will have a bz-m(F) endosperm and a bz-s embryo lacking *Ac*.

Other postmeiotic transposition events not diagrammed here will produce nonconcordant kernels with a bz-m(F) endosperm and a *bz-m2(Ac)* embryo and, as mentioned earlier, others will also contribute to the concordant class. Notice in Table 3 the high proportion of concordant derivatives carrying unlinked tr*Ac*s. The greater the contribution to the concordant class of postmeiotic events relative to meiotic events, the greater the proportion of unlinked tr*Ac*s to linked tr*Ac*s will be since there is no intervening meiosis to segregate out *Ac*s that have transposed to unlinked sites.

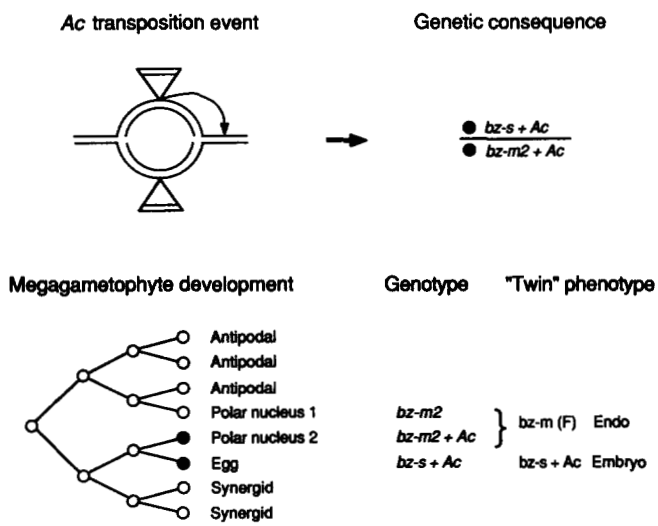

FIGURE 7.—Model to explain the origin of nonconcordant bz-m(F) derivatives where the embryo carries a *bz-s* allele and a tr*Ac*.

An interesting point to consider in the analysis of the distribution of linked to unlinked receptor sites for tr*Ac*s is whether their ratio changes in different cell divisions. In an attempt to obtain an answer to this question one can make the following comparison. Since *bz-s* derivatives occur as single kernels in *bz-m2* ears, they can be considered to arise at or shortly before meiosis. (A transposition event occurring postmeiotically cannot result in a kernel with a bronze phenotype because the two polar nuclei have different cell lineages.) Conversely, noncorresponding kernels must originate from gametophytic transposition events. Therefore, a comparison of the ratio of linked to unlinked tr*Ac*s in these two classes might reveal if large differences occur in the distribution of *Ac* receptor sites between meiosis and gametophytic mitoses.

Among the 49 *bz-s* derivatives carrying a tr*Ac*, *Ac* was found to be unlinked in 20 (Table 1). One can expect an equal number of unlinked tr*Ac*s to have been lost by meiotic segregation. Therefore, at meiosis, the proportion of receptor sites unlinked to the donor site would be 40/69 or 58%. Though the number of nonconcordant bz-m(F) derivatives carrying *bz-s* and a tr*Ac* was small (Table 3), examination

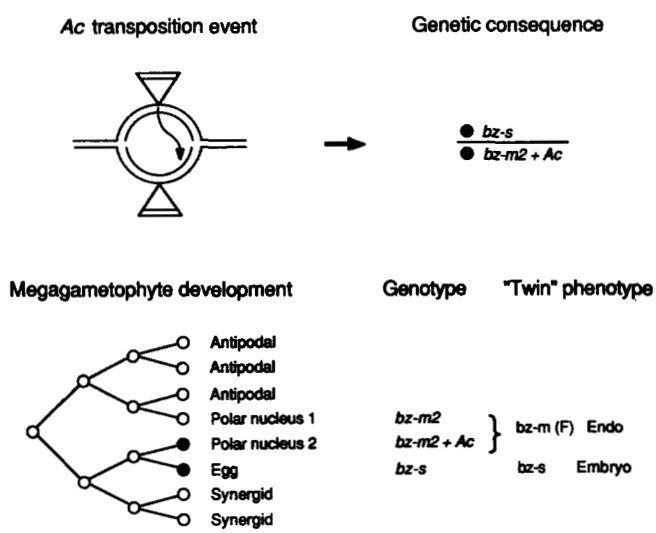

FIGURE 8.—Model to explain the origin of nonconcordant bz-m(F) derivatives where the embryo carries a *bz-s* allele without a tr*Ac*.

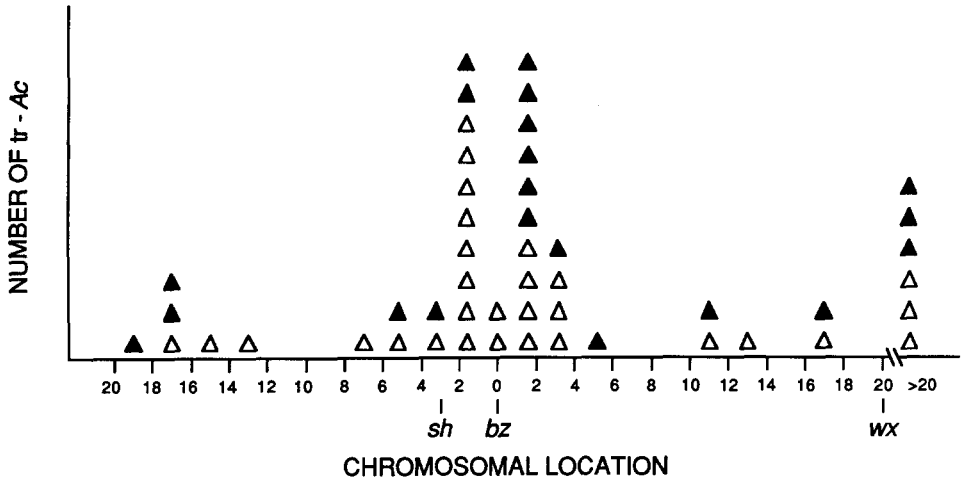

FIGURE 9.—Cumulative distribution of tr*Ac*s among bz-s and bz-m(F) selections from *bz-m2(Ac)*. Open triangles: bz-s selections; solid triangles: bz-m(F) selections.

TABLE 5

Distribution of linked tr-*Ac*s among bz-s and bz-m(F) selections from *bz-m2(Ac)*

	bz-Ac distance (cM)				
Derivative	<1	1–5	5–20	>20	Total
bz-s	11	8	7	3	29
Fraction	0.38	0.28	0.24	0.10	1.0
bz-m(F)	4	8	5	3	20
Fraction	0.20	0.40	0.25	0.15	1.0

of the distribution of linked and unlinked receptor sites for the tr*Ac*s is instructive, nevertheless. In this group there is no loss of tr*Ac*s by meiotic segregation. Of 8 individuals in the class, 5 or 63% had an unlinked *Ac*. Thus, there is no indication in these data that the ratio of linked to unlinked receptor sites changes between meiosis and the ensuing postmeiotic mitoses.

As pointed out in RESULTS, the clustering of tr*Ac*s around the *bz* donor site appears to be different in bz-s and bz-m(F) selections from *bz-m2(Ac)*. This difference is made more obvious if one disregards direction of transposition and pools the transposed *Ac*s into classes covering progressively larger map distances away from *bz*, as has been done in Table 5. Very close transpositions, those occurring to sites within 1 cM of *bz*, are about twice more frequent in the bz-s class than in the bz-m(F) class. This difference can be accounted for in the GREENBLATT and BRINK (1962) model if very short-range transpositions at or shortly after meiosis, tend to be intrachromatid transpositions, *i.e.*, from a donor site to a replicated site in the same chromatid. Such transpositions would produce bz-s derivatives with very closely linked tr*Ac*s, but would not produce bz-m(F) derivatives. GREENBLATT (1984) has raised the important question of whether the means of uncovering the transpositions could influence the recovery of receptor site locations. At least for *bz*, this is clearly the case.

Finally, though the above analysis of the distributions of tr*Ac*s among the two types of selections reveals that there may be differences, pooling of the data for the 49 linked tr*Ac*s serves to emphasize the general features of *Ac* transposition to sites linked to *bz*: the receptor sites tend to be clustered on either side of the donor site and transposition appears to be bidirectional and nonpolar (Figure 9).

In terms of their implication on strategies for tagging genes with *Ac*, the *bz* locus data support the existing *P* locus data (VAN SCHAIK and BRINK 1959; GREENBLATT and BRINK 1962; GREENBLATT 1984) in showing a pronounced preference for short range transpositions of *Ac*. Therefore, it is important to initiate an *Ac* mutagenesis experiment with an *Ac* element closely linked to the desired target locus. This can be presently achieved in corn through the use of translocation chromosomes carrying *Ac* (*e.g.*, GREENBLATT's derivation of *mR-nj*, cited in BRINK and WILLIAMS 1973) and it may soon be possible in dicots through the use of transformed lines carrying easily scorable *Ac*-mutable genes, such as the green-white variegating *SPT::Ac* (streptomycin phosphotransferase), at appropriate locations in the genome (JONES *et al.* 1989).

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