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Sequences Related to the Maize Transposable Element *Ac* in the Genus *Zea*

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Summary. In light of previous data, which suggested that *Ac*-like sequences might have undergone a significant radiation in the recent past, I examined the copy number of *Ac*-like sequences in representatives of all the *Zea* taxa, both maize and teosinte. The maize and teosinte samples contained approximately equal numbers of *Ac*-like sequences. Few *Ac*-like sequences were in unmethylated regions of DNA. Unmethylated elements were distributed randomly among both maize and teosinte lines. The appearance in a line of a discrete band resulting from digestion with one methylation-sensitive restriction enzyme was correlated with the appearance of discrete bands with other methylation-sensitive bands. This suggests that individual *Ac*-like elements are occasionally demethylated in many sites. No unmethylated element having restriction fragments of the lengths predicted from the published *Ac* sequence was seen in the approximately 326 elements examined.

Key words: Maize — Teosinte — *Ac* — Transposable elements

Introduction

Recent work has shown that *Ac*-like sequences, DNA sequences that hybridize to probes from the maize transposable element *Ac*, are not randomly distributed in the genome of maize. Instead, a large number of these sequences are found in a group on chromosome 4 (Johns et al. 1990). The rest of the *Ac*-like sequences are scattered throughout the genome, usually in different locations in different lines. Genetic evidence implies that *Ac* elements increase in number by an average of 1.44 elements per transposition, and that *Ac* usually transposes to a linked site (Greenblatt 1966, 1968, 1974, 1984; Chen et al. 1987). Using this information about *Ac* transposition, the observed distribution of *Ac*-like sequences could be modeled as the result of 10 or fewer transpositions by the descendants of a single original element on chromosome 4.

The small number of postulated transposition events might imply a recent origin for *Ac*, as too great an interval between transpositions would allow mutational inactivation of all copies of *Ac*. Although most maize lines do not contain active *Ac* elements, *Ac* has been activated by artificial means on several occasions (McClintock 1947, 1951; Peschke et al. 1985; Rhoades and Dempsey 1982). Also, a naturally occurring form of *Ac* exists at the P^{vv} locus (Barclay and Brink 1954). Thus, structurally intact but genetically inactive forms of *Ac* must exist in at least some maize lines. A limited amount of published data indicates that *Ac*-like sequences are found in many maize lines (e.g., Fedoroff et al. 1983; Chen et al. 1987; Wessler 1988).

Domesticated maize is quite different in flower structure and general appearance from its probable ancestor, teosinte. Teosinte is a common name that is applied to several distinct wild species of *Zea*. Based on its appearance, the teosintes were originally classified into a separate genus, *Euchlaena* (Iltis and Doebley 1984). However, teosinte and maize interbreed easily, and some forms of teosinte and maize produce fully fertile offspring (Collins and Kempton 1920). A recent scheme puts maize and several teosinte types into the same species, *Zea mays*, whereas other teosinte types belong to sepa-

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rate species within the genus *Zea* (Doebley and Iltis 1980; Iltis and Doebley 1980). Recent molecular comparisons, including isozymes and chloroplast DNA polymorphisms, confirm the close relationship between maize and teosinte (Doebley et al. 1984, 1987). Restriction sites in chloroplast DNA in some *Zea mays* ssp. *mays* (maize) lines were identical to those found in some *Zea mays* ssp. *parviglumis* and *Zea mays* ssp. *mexicana* (both teosintes) lines. Thus, despite major morphological differences, maize and teosinte are very closely related.

The maize ear lacks a seed dispersal mechanism, causing maize to be a strictly domesticated species. There is little evidence for human habitation in the New World before 15,000 years ago, suggesting that maize was derived from one of the teosinte species within the past 15,000 years (Iltis and Doebley 1984). It is reasonable to expect that some major genetic event(s) occurred during domestication, but randomly chosen isozymes and chloroplast DNA sequences do not reflect this event (Doebley et al. 1984, 1987). Thus, the suggestion that *Ac* might have existed as a transposable element for only a short time needs to be investigated in terms of the recent maize–teosinte split.

I have found that the copy number and distribution of *Ac*-like sequences among members of the genus *Zea* do not suggest any major recent evolutionary events. The *Ac*-like sequences are found in a similar number in all taxa investigated. Most of these sequences are heavily methylated. Those few sequences that are not methylated are found equally in maize and teosinte, and none of the undermethylated sequences has restriction sites similar to that of the standard *Ac* element (Fedoroff et al. 1983; Pohlman et al. 1984). This implies that genetically active *Ac* elements are uncommon among *Zea* lines.

Materials and Methods

The genus *Zea* has been classified into two sections. Section *Zea* contains a single species, the highly polymorphic *Z. mays*, comprised of the subspecies *Z. m. mays* (corn or maize), *Z. m. mexicana* (large-spikeleted teosinte, with races Chalco, Central Plateau, and Nobogame), and *Z. m. parviglumis* (small-spikeleted teosinte, with varieties *parviglumis* and *huehuetenangensis*). Section *Luxuriantes* contains the species *Zea luxurians* (Guatemalan teosinte), *Zea perennis* (tetraploid perennial teosinte), and *Zea diploperennis* (diploid perennial teosinte) (Doebley and Iltis 1980; Iltis and Doebley 1980). Maize thus refers to the single subspecies *Z. m. mays*, characterized by polystichous ears and soft, usually tiny glumes on the kernels. Teosinte refers to any of several species or subspecies, all of which have distichous disarticulating ears and kernels covered by hard glumes. Seed from each of the identified teosinte species and subspecies was obtained from Dr. John Doebley (University of Minnesota) or from the Regional Plant Introduction Station (Ames, Iowa). Maize lines were obtained from a variety of sources, including the Maize Genetics Cooperative, Lifaco Seed Co., Dr. Michael Murray (Agrigenetics), and Dr. Arthur Hooker. Table 1 lists the lines examined and their origins.

Plants were grown for 6 weeks in 20-cm pots in the greenhouse and then harvested into liquid nitrogen. DNA was prepared from the leaves of individual plants by the method of Saghai-Maroof et al. (1984). Southern blots were performed as described in Johns et al. (1990). Briefly, DNA was digested with restriction endonucleases according to the manufacturer's recommendations, electrophoresed, and blotted as described in Maniatis et al. (1982) onto Zeta-probe (Bio-Rad) nylon membranes. After hybridization the membranes were hybridized with the ^{32}P-labeled pEH0.7 probe, consisting of the 0.7-kb internal *Hin*dIII–*Eco*RI fragment of pAc9 (Fedoroff et al. 1983). The hybridized filters were washed at a series of increasing stringencies, culminating in a final wash in 0.1× SSPE (1× SSPE = 0.18 M NaCl, 0.01 M Na-phosphate, 1 mM EDTA), 0.75% SDS at 65°C for 60 min. Washed blots were exposed to x-ray film for 4 days.

Results

Copy Number

The number of bands seen on Southern blots with *Eco*RI-digested DNA from each line is given in Table 2 and illustrated in Fig. 1. The band numbers in Table 1 are unlikely to equal the copy number of *Ac*-like sequences for several reasons. First, the maize and teosinte samples are not equivalent. The maize lines represent either inbred lines, which are completely homozygous, or cultivars, which are at least partly inbred. Thus, the band number in maize should be approximately equal to the copy number per haploid genome. In contrast, the teosintes are accessions from the wild and are probably heterozygous at many loci. How this affects the relationship between band number and copy number depends on the transposition rate of *Ac*-like sequences and on the level of restriction site polymorphism near each element. However, the band number should be between one and two times the haploid genome copy number.

Another complicating factor is the difficulty in getting an exact count of the bands. The band numbers shown in Table 2 are the rounded off averages of 1–3 individual plants. All visible bands were counted, and extra-heavy bands were counted as two bands (doublets) only if the two bands were visibly separated. This method necessarily undercounts the copy number, because two bands of identical size will be counted as only one, and the chance of undercounting increases with increased copy number.

A third possible source of bias in equating band number with copy number is the choice of restriction enzyme used. To control for this factor, 16 of these lines were also digested with *Dra*I and with *Bam*HI. The number of bands seen with either of these enzymes differed by no more than two from the number of *Eco*RI bands, suggesting that the

Table 1. Plants analyzed

Line or acquisition		Source	Collection
Section *Luxuriantes*			
684	*Z. perennis*	Collins	s.n.[a]
669	*Z. diploperennis*	Guzman	777
670	*Z. luxurians*	Iltis	G-5
673	*Z. luxurians*	Iltis	G-42
685	*Z. luxurians*	Iltis	G-38
Section *Zea*			
	Z. mays		
672	subsp. *parviglumis*		
	var. *huehuetenangensis*	Iltis	G-120
667	subsp. *parviglumis*		
	var. *parviglumis*	Iltis and Nee	1480
668	var. *parviglumis*	Iltis and Cochrane	308
674	var. *parviglumis*	Kato	K-77-13
679	var. *parviglumis*	Benz	967
680	var. *parviglumis*	Benz and Solheim	890b
683	var. *parviglumis*	Beadle (El Salado)	s.n.
324	subsp. *mexicana*	RPIS-A[b]	384062
326	subsp. *mexicana*	RPIS-A	384074
327	subsp. *mexicana*	RPIS-A	384069
328	subsp. *mexicana*	RPIS-A	355921
675	subsp. *mexicana*	Doebley	625
677	subsp. *mexicana*	Doebley	642
678	subsp. *mexicana*	Doebley	481
681	subsp. *mexicana*	Beadle	s.n.
682	subsp. *mexicana*	Puga	11066
	subsp. *mays*		
604	Lady Finger Pop	MGC[c]	77-099
609	Red South American Pop	MGC	81-1628
610	Argentine Pop	MGC	83-871
612	Knobless Willur's Flint	MGC	81-1663
613	Tama Flint	MGC	82-18964
614	Papago Flour Corn	MGC	81-1588-3
615	Hulless Pop	MGC	81-1641
616	Super Gold Pop	MGC	82-7594
617	Tama Flint Knobless	MGC	82-759-4
618	Black Max Sweet W/Bs	MGC	84-675
620	Gourdseed	MGC	81-1668
622	Maize Chapalote	MGC	83-921-1
623	Ohio Yellow Pop	MGC	81-1634
691	Hickory King	Sisco	84:26
1213	A632	Hooker	s.n.
1221	B37	Hooker	87:1199B
901	B73	Lifaco	s.n.
1230	Oh43	Hooker	s.n.
1226	R109B	Hooker	87:1482-1
1235	W153R	Hooker	s.n.
1245	W117	Agrigenetics	220
1246	H93	Agrigenetics	160
1247	Mp305	Agrigenetics	130
1248	CI31A	Agrigenetics	282

[a] Sine numero, not numbered
[b] Regional Plant Introduction Station—Ames
[c] Maize Genetics Cooperative

choice of *Eco*RI did not significantly affect the number of visible bands.

Figure 1 shows an example of each of the identified *Zea* species and subspecies digested with *Eco*RI and probed with an internal *Ac* fragment. With each line, most of the hybridizing bands are of approximately the same intensity and number. Occasional fainter bands appear in some lanes; these bands probably represent either *Ac*-like sequences with weak homologies to the probe or elements having only a small region of overlap with the probe. A larger study (Table 2) shows that there are an average of 8.6 ± 2.1 bands hybridizing to this probe among the 42 lines tested. When maize is considered sep-

Table 2. Number of bands per line or acquisition

Number	Line name	*Eco*RI	*Pst*I	*Pvu*II
604	*Z. m. mays*	7	0	1
609	*Z. m. mays*	nd	0	0
610	*Z. m. mays*	8	1	1
612	*Z. m. mays*	7	0	0
613	*Z. m. mays*	8	0	0
614	*Z. m. mays*	8	0	0
615	*Z. m. mays*	5	0	0
616	*Z. m. mays*	8	0	0
617	*Z. m. mays*	nd	0	0
618	*Z. m. mays*	8	0	1
620	*Z. m. mays*	10	1	1
622	*Z. m. mays*	7	0	0
623	*Z. m. mays*	5	0	1
691	*Z. m. mays*	7	0	0
1245	*Z. m. mays*	8	nd	nd
1246	*Z. m. mays*	9	nd	nd
1247	*Z. m. mays*	7	nd	nd
1248	*Z. m. mays*	6	nd	nd
A632	*Z. m. mays*	7	nd	nd
B37	*Z. m. mays*	11	nd	nd
B73	*Z. m. mays*	9	nd	nd
Oh43	*Z. m. mays*	5	nd	nd
R109B	*Z. m. mays*	9	nd	nd
W153R	*Z. m. mays*	7	nd	nd
324	*Z. m. mexicana*	8	0	0
326	*Z. m. mexicana*	9	0	0
327	*Z. m. mexicana*	11	2	0
328	*Z. m. mexicana*	12	nd	nd
675	*Z. m. mexicana*	7	0	0
677	*Z. m. mexicana*	13	1	2
678	*Z. m. mexicana*	7	0	0
681	*Z. m. mexicana*	12	1	3
682	*Z. m. mexicana*	8	0	1
667	*Z. m. parviglumis*	10	0	1
668	*Z. m. parviglumis*	8	0	1
674	*Z. m. parviglumis*	8	0	2
679	*Z. m. parviglumis*	nd	0	0
680	*Z. m. parviglumis*	9	0	0
683	*Z. m. parviglumis*	10	0	1
672	*Z. m. huehuetenangensis*	7	nd	0
670	*Z. luxurians*	10	0	0
673	*Z. luxurians*	11	0	0
685	*Z. luxurians*	11	nd	0
684	*Z. perennis*	11	1	3
669	*Z. diploperennis*	14	0	0

nd, no data

arately from the teosintes, the maize average is 7.8 ± 2.0 bands per line and the teosinte average is 9.8 ± 2.0 bands per line. When the *Luxuriantes* section teosintes are ignored, the *Z. mays* teosintes have an average copy number of 9.3 ± 1.9 bands per line.

The data in Table 2 show that all of the *Zea* taxa contain similar numbers of *Ac*-like sequences. Given the difficulties in equating band number with copy number, the slightly greater number of bands in the teosintes compared to the maize lines is probably not significant.

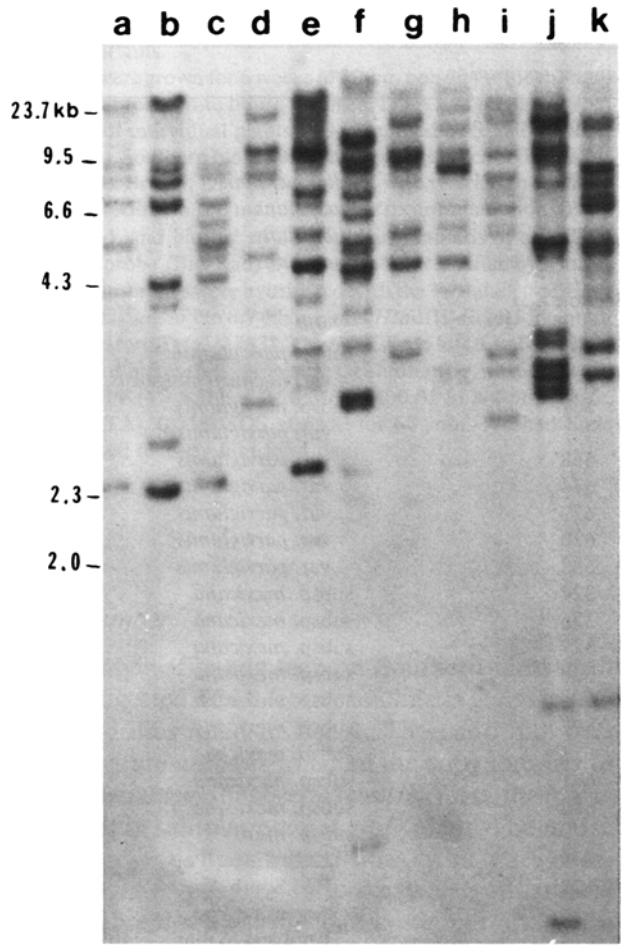

Fig. 1. DNA blot hybridizations of *Zea* lines digested with *Eco*RI using an internal fragment of *Ac* as a probe. a, *Zea mays mays* B73; b, *Z. m. mays* Super Gold Pop; c, *Z. m. mays* Tama Flint; d, *Z. m. parviglumis* var. *huehuetenangensis*; e, *Z. mays parviglumis* var. *parviglumis*; f, *Z. mays mexicana* race Chalco; g, *Z. m. mexicana* race Central Plateau; h, *Z. m. mexicana* race Nobogame; i, *Z. luxurians*; j, *Z. diploperennis*; k, *Z. perennis*.

Methylation

Ac elements can exist in two forms: the genetically active form, which is capable of transposition, and the inactive inert form. Specific cytosine residues are unmethylated in active *Ac* elements but methylated in inactive forms (Schwarz and Dennis 1986; Chomet et al. 1987) This difference in cytosine methylation usually extends to the surrounding DNA, which is hypomethylated near active *Ac* elements but hypermethylated near the more common inactive elements (Chen et al. 1987). Methylation of specific regions is usually assessed by sensitivity to digestion by restriction endonucleases such as *Pst*I or *Pvu*II, which will not cut DNA if their recognition sites contain 5-methylcytosine.

Figure 2A shows a selection of *Zea* lines digested with *Pst*I, which does not cut within the standard *Ac* element. Most lines contain only a smear of high

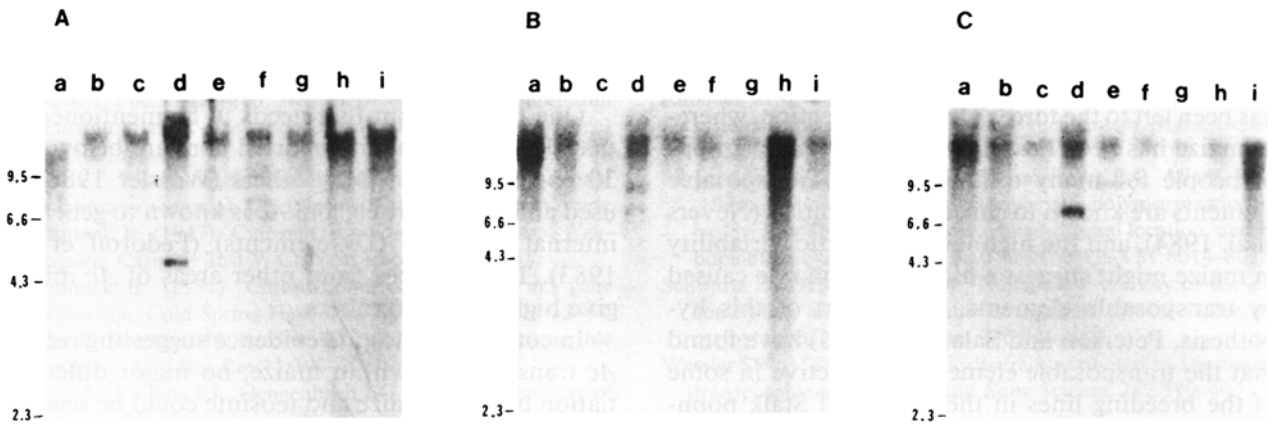

Fig. 2. Blot hybridizations of *Zea* lines probed with *Ac* probe. **A** *Pst*I digestion; **B** *Pvu*II digestion; **C** *Sst*II digestion. a, *Zea mays mays* B73; b, *Z. m. parviglumis* var. *huehuetenangensis*; c, *Z. m. parviglumis* var. *parviglumis*; d, *Z. m. mexicana* race Chalco; e, *Z. m. mexicana* race Central Plateau; f, *Z. m. mexicana* race Nobogame; g, *Z. luxurians*; h, *Z. diploperennis*; i, *Z. perennis*.

molecular weight DNA, with no sharp bands to indicate digestion of specific sites near a specific *Ac*-like sequence. All lines contain multiple copies of hypermethylated *Ac*-like sequences. Occasional sharp bands do appear, as in lane 4 (*Z. m. mexicana* race Chalco). Table 2 lists the number of *Pst*I bands seen for each line. Equating the number of discrete bands seen below the high molecular weight smear with the number of unmethylated *Ac*-like sequences is affected by the possible existence of unmethylated regions that lack closely spaced *Pst*I sites flanking the element. The band for such an element would be lost in the high molecular weight smear. Thus, the number of hypomethylated *Ac*-like sequences is underestimated in Table 2. Most of the *Zea* lines do not have unmethylated *Ac*-like sequences, and no line has more than two discrete *Pst*I bands. Also, hypomethylated *Ac*-like sequences are found in about equal frequency in maize and the teosintes.

Similar results were obtained using *Pvu*II, another methylation-sensitive enzyme. Once again, all of the lines showed a large smear of high molecular weight DNA indicating the presence of methylated *Ac*-like sequences. Distinct bands appeared in only 13 of the 34 lines surveyed, and maize and teosinte lines were about equally likely to have a hypomethylated *Ac*-like sequence.

*Pvu*II has two recognition sites within the standard *Ac* element. Active *Ac*s were first molecularly differentiated from inactive elements by the presence of a distinct 2.5-kb *Pvu*II band in active lines only (Fedoroff et al. 1983). The six active *Ac* elements sequenced to date all generate a 2.5-kb band upon *Pvu*II digestion (Wessler 1988). None of the discrete *Pvu*II bands seen in the 34 lines surveyed was of the 2.5-kb size expected from an intact *Ac* element; hypomethylated *Ac* elements of the standard sequence must be quite rare in *Zea* lines.

The presence of a *Pst*I band in a line is often correlated with the presence of a *Pvu*II band. This suggests that individual elements are probably occasionally demethylated at many sites, allowing digestion by both enzymes. As an example, Fig. 2 shows a selection of *Zea* lines digested with *Pst*I, *Pvu*II, and *Sst*II, which is also methylation-sensitive. In all three cases, lane d, with *Z. m. mexicana* race Chalco, has a discrete band. Other cases where a hypomethylated band appears with one enzyme but not with another might be explained by unmethylated but large bands lost in the high molecular weight smear. These data are consistent with studies of methylation and demethylation of active *Ac* elements (Chen et al. 1987; Chomet et al. 1987), in which active *Ac* elements are demethylated at many sites.

Discussion

Anecdotal evidence has suggested that *Ac*-like sequences are found in many *Zea* lines. I have shown that *Ac*-like sequences are indeed present in representatives of all the taxa of *Zea*. *Ac* is thus probably an ancient component of the *Zea* genome and not a recent acquisition.

If a dramatic radiation of *Ac* elements had occurred simultaneously with the differentiation of maize from its ancestral teosinte, which is one possible interpretation of the data of Johns et al. (1990), I might have found large differences in copy number between maize and some or all of the teosintes. The insignificant differences in copy number found are not consistent with this hypothesis. It will be necessary to search for other explanations for the nonrandom chromosomal distribution of *Ac*-like sequences in maize lines.

It is remarkable that maize and the teosintes all have similar copy numbers—there is less than a

threefold difference between the lowest and the highest lines. Maize has had a very different history than teosinte in the last 10,000 years. Teosinte, a weed, has been left to the forces of natural selection, whereas maize has been heavily selected by many groups of people for many different traits. Transposable elements are known to cause gene mutation (Nevers et al. 1984), and the high level of genetic variability in maize might suggest a high mutation rate caused by transposable elements. In support of this hypothesis, Peterson and Salamini (1986) have found that the transposable element *Uq* is active in some of the breeding lines in the Iowa Stiff Stalk population, but none of the inbreds derived from this line carry it. Variation is expected and acceptable in the breeding lines, but the derived inbreds are selected for uniformity. Their studies identified only genetically active *Uq* elements, but the same principle should apply to inactive transposable elements that occasionally become active. It is possible that the effects of breeding for and against variability have balanced out for *Ac*-like sequences, or it may be that *Ac* is simply not a very important element for generating genetic variability in maize.

The DNA within and near most *Ac*-like sequences is hypermethylated. In plants, cytosine residues in the sequences of CG or CNG (where N is any nucleotide) are often methylated (Gruenbaum et al. 1981). Near genes or active transposable elements, cytosine methylation is reduced (Chen et al. 1987; Antequera and Bird 1988). Hypermethylation of most of the *Ac*-like sequences in all the *Zea* lines tested suggests that they are not active.

All active *Ac* elements that have been sequenced have virtually identical sequences, and they are all hypomethylated, giving discrete 2.5-kb *Pvu*II bands (Wessler 1988). The fact that I found no unmethylated *Pvu*II bands of this size implies that active *Ac* elements are very uncommon in unselected *Zea* acquisitions. There were no reasonable candidates for an active element among the 326 *Ac*-like sequences (*Eco*RI bands) examined. For this reason, transposition of *Ac*-like sequences is not likely to be frequent in *Zea* considered as a whole, although individual plants or lines with active *Ac* elements undoubtedly have a higher transposition rate. However, *Ac* has been activated on several occasions, originally by McClintock (1947), and then independently by Rhoades and Dempsey (1982) and by Peschke et al. (1985). These artificial activations give weight to the hypothesis that *Ac* can become active on occasion in natural populations. To be activatable in several different lines of modern maize implies that at least some of the *Ac* elements in these lines have not been irreversibly inactivated by mutation. It seems likely that in some lines, *Ac* elements are maintained intact but hypermethylated and inactive. The mechanisms by which these elements can become demethylated and active are mostly unknown at present.

One further point that needs to be mentioned is that the copy numbers reported here are below the 30 copies mentioned by others (Wessler 1988). I used an internal probe, and *Ac* is known to generate internal deletions (*Ds* elements) (Fedoroff et al. 1983). Thus, probes from other areas of *Ac* might give higher copy numbers.

In conclusion, despite evidence suggesting recent *Ac* transposition within maize, no major differentiation between maize and teosinte could be seen in *Ac* copy number or methylation state. My data support that of Doebley et al. (1984, 1987), showing no clear molecular differences between maize and the more closely related teosintes.

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