Genet. Res., Camb. (1990), **55**, *pp.* 71–80 *With 3 text-figures Printed in Great Britain*

Non-random chromosomal distribution of *Ac*-like sequences in inbred maize

MITRICK A. JOHNS*, SUSAN I. FUERSTENBERG† AND CHERYL A. HENNELLY
Plant Molecular Biology Center, Northern Illinois University, DeKalb, Illinois 60115, USA

(*Received 25 July 1989 and in revised form 13 November 1989*)

Summary

We have searched for evidence of historical transpositions of *Ac*-like sequences in four standard maize lines using the recombinant-inbred mapping technique. Thirty restriction fragments were mapped using *Eco*R I, *Eco*R V, and *Hin*d III. The four inbreds contained 24 fragments which mapped to independent sites within each line; the other 6 fragments probably represented multiple mappings of the same element. Possible allelism between lines reduced this number to a minimum of 15 different sites containing *Ac*-like elements. The distribution of these sequences does not fit the expected Poisson distribution; instead, an unusually large number of these elements were found on chromosome 4. The other sequences were scattered randomly throughout the genome. With few exceptions, each line had sequences in different locations; however, the overall distribution of *Ac*-like sequences was similar for all lines. The non-random distribution of *Ac*-like sequences suggests that they have undergone a limited number of transpositions in maize; the distribution is incompatible with either complete immobility or frequent transposition.

1. Introduction

The autonomous transposable element *Ac* was discovered by McClintock (1947, 1951) in a maize line that had been subjected to a cycle of repeated chromosome breakage and fusion. This line had previously been genetically stable. Most of the *Ac* elements studied since that time have been derived from this original element, or from the identical, naturally occurring *Mp* element found at the P^{vv} locus (Barclay & Brink, 1954). *Ac* is defined genetically as having the ability to catalyze the transposition of non-autonomous *Ds* elements. Using this criterion, Peterson & Salamini (1986) have shown that *Ac* is not present in a number of standard maize inbred lines.

Ac elements have been generated in previously non-*Ac* lines by several experimental manipulations. Rhoades & Dempsey (1982) found the very similar *Ac2* element in a 'High Loss' line that had a large heterochromatic knob on chromosome 10 that caused repeated chromosome breaks due to late replication of heterochromatin. Peschke, Phillips & Gengenbach (1987) were able to activate *Ac* by regenerating maize plants from tissue culture. Other transposable elements have been activated by X-rays, gamma-rays, chemical mutagens, and virus infections (Burr, Archer & Burr, 1987; Sprague & McKinney, 1966; Mottinger, Johns & Freeling, 1984; Doerschug, 1973; Walbot, 1986). McClintock (1978, 1984) has proposed that transposable elements are liberated from a quiescent state in response to 'genomic stress' as an evolutionary defense mechanism.

After *Ac* was cloned (Fedoroff, Wessler & Shure, 1983), it became apparent that *Ac*-like sequences were present in many or all maize lines. This group of sequences consists of intact *Ac* elements, *Ds* elements whose transposition requires activation by *Ac*, and possibly other related but non-transposable sequences. Some of these *Ac*-like sequences apparently differ from *Ac* only by having a large number of methylated cytosine residues. In lines showing reversible inactivation of *Ac*, hypermethylation of cytosines is well correlated with inactivation (Schwartz & Dennis, 1986; Chomet, Wessler & Dellaporta, 1987). Another maize transposable element, Mutator, has also been shown to be inactivated by cytosine methylation (Chandler & Walbot, 1986; Bennetzen, 1987; Chandler, Talbert & Raymond, 1988).

DNA methylation is heritable, but it is probably not as stable as the nucleotide sequence. It seems possible that *Ac* could occasionally become active in normal, untreated maize. This would result in transposition, and over time, the positions of *Ac*-like

* Corresponding author.
† Current address: Department of Plant Biology, University of Minnesota, St Paul, MN 55108. USA

elements among maize lines would differ from one another. Alternatively, if *Ac* could only be activated by extraordinary events, its positions would be the same in virtually all lines.

The development of recombinant inbred (RI) lines (Burr *et al.* 1988) has made it possible to map specific DNA sequences quickly and accurately. Using these lines, we have mapped the chromosomal locations of 24 *Ac*-like sequences in the four RI parental lines. The elements are distributed in a non-random fashion, with many of them located on chromosome 4. Very few cases of elements from different lines mapping to the same location other than on chromosome 4 were noted. The clustering of these elements is consistent with the occasional activation and transposition of *Ac* in these lines, and with the previously observed behaviour of *Ac* during transposition (Greenblatt, 1966, 1968, 1974, 1984; Chen, Greenblatt, & Dellaporta, 1987; Dooner & Belachew, 1989).

2. Materials and methods

(i) *Strains*

The entire set of recombinant inbred lines, consisting of 48 TXCM lines and their parents T232 and CM37, and 41 COXTx lines and their parents CO159 and Tx303, was obtained from Dr Benjamin Burr, Brookhaven National Laboratory. These lines have been described (Burr *et al.* 1988), as have the original lines and their F2 populations (Stuber & Edwards, 1986; Edwards, Stuber & Wendel, 1987). Briefly, the inbreds used in each cross, T232 × CM37 and CO159 × Tx303, were chosen to maximize differences at allozyme loci and in agronomic and morphological traits. All four lines are publicly available American Corn Belt inbreds, but CO159 and CM37 are northern lines while T232 and Tx303 are southern lines. The large number of phenotypic differences between the lines implies that they are not closely related, and they are not known to share any common parents.

The recombinant-inbred lines were created by six generations of self-pollination from the F1 hybrids of T232 × CM37 and CO159 × Tx303. This procedure resulted in a set of lines, each of which was homozygous for a different set of parental alleles at most loci. However, there is an average residual heterozygosity of 7·5% for all loci. Each RI line is homozygous for a unique set of parental alleles, and each locus has a unique allelic distribution among the RI lines. This fact can be exploited for mapping new loci.

(ii) *Data analysis*

Data, in the form of a list of which parental allele (presence or absence of a band) was present in each RI line, were analysed by comparing them to the RI database provided by B. Burr. This database lists the allelic distribution among the RI lines of about 200 loci. Every locus has a unique distribution of parental alleles among the RI lines, so a new locus can be mapped by comparing the distribution of its alleles in the RI lines to those already present in the database: a close match indicates linkage. Virtually every locus is within 20 map units of another. Data comparisons were performed using a program written for Lotus 1-2-3 spreadsheet software (Lotus Development Corp.), based on the algorithm given in Burr *et al.* (1988).

(iii) *DNA preparation*

Plants were grown in 20 cm pots for 6 weeks, then harvested into liquid nitrogen and lyophilized. The dried plant material was powered in a mill, and the DNA was extracted by the method of Saghai-Mahroof *et al.* (1984).

(iv) *Restriction enzyme digestion, electrophoresis, blotting and hybridization*

Samples of 15 μg of DNA were digested with 50 units of restriction enzyme for 4 h under conditions recommended by the manufacturer (BRL). The digested DNA was electrophoresed through 0·7% agarose gels in standard Tris-borate-EDTA buffer (Maniatis, Fritsch & Sambrook, 1982). The gels were treated with depurination, denaturation and neutralization solutions, then blotted for 18 h to Zeta-probe nylon membranes (Bio-rad) according to Southern (1975). After blotting, the membranes were baked at 80 °C for 2 h, than prehybridized for 2–4 h at 42 °C in a solution of 6 × SSPE, 1% SDS, 10 × Denhardt's, 50 μg/ml denatured salmon sperm DNA. Hybridization was performed using 20–50 × 10^6 cpm of probe labelled to a specific activity of at least 3 × 10^8 cpm/μg by the random priming method of Feinberg & Vogelstein (1983). The probe used was the 0·7 kb *Eco*R I–*Hin*d III internal fragment of *Ac*, derived from pAc9 (Fedoroff, Wessler & Shure, 1983). This fragment contains a *Bgl* I site but not an *Sph* I site. Each 20 × 20 cm blot was hybridized in 25 ml of 6 × SSPE, 1% SDS, 50% formamide, 50 μg/ml salmon sperm DNA containing the probe. After hybridization, the blots were washed in 6 × SSPE, 0·2% SDS at room temperature for 30 min, then 1 × SSPE, 0·75% SDS at 42 °C for 30 min, and finally in 0·1 × SSPE, 0·75% SDS at 65 °C for 60 min. The stringency of the final wash was calculated to be T_m − 10 °C. Blots were then exposed to Kodak XAR-5 film for 3–6 days at −70 °C using a DuPont Cronex Lightening-Plus intensifying screen.

3. Results

(i) *Mapping of* Ac*-like elements using RI lines*

Each RI parent had a unique set of *Ac*-containing restriction fragments (Fig. 1). Most of the bands on

https://doi.org/10.1017/S0016672300025295 Published online by Cambridge University Press

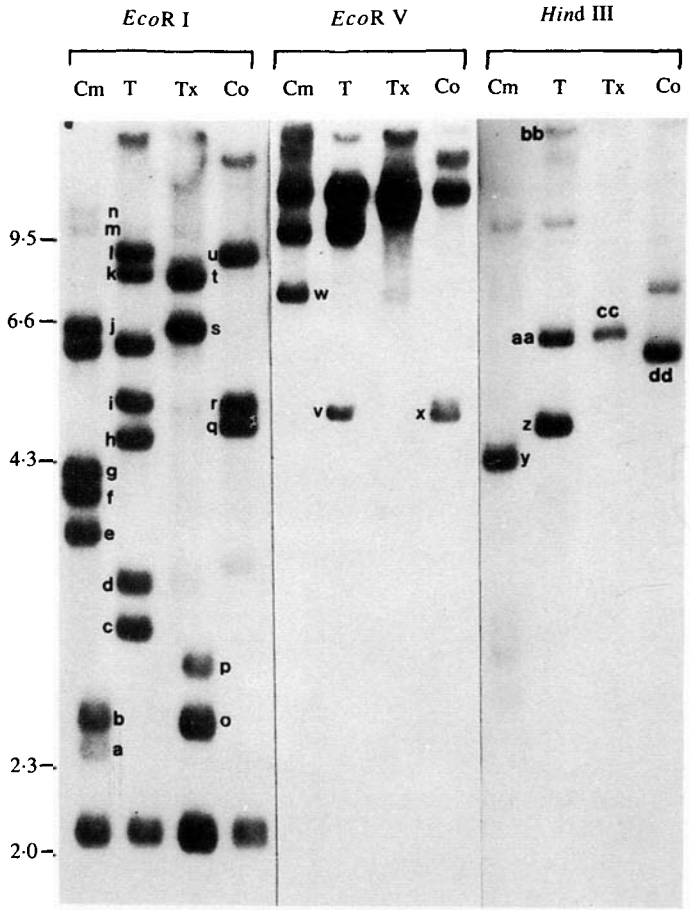

Fig. 1. *Ac*-like sequences in RI progenitors digested with the restriction enzymes used for mapping. The autoradiograms were produced as described in the Methods section. Size standards derived from *Hind* III-digested DNA from bacteriophage lambda are indicated. The mapped bands are labeled as follows, with approximate sizes in parentheses: a, E1-1a (2·6 kb); b, E1-2a (2·8 kb); c, E1-3a (3·2 kb); d, E1-4a (3·5 kb); e, E1-5a (3·8 kb); f, E1-6a (4·1 kb); g, E1-7a (4·3 kb); h, E1-8a (4·6 kb); i, E1-9a (5·2 kb); j, E1-10a (6·6 kb); k, E1-11a (8·2 kb); l, E1-12a (9·0 kb); m, E1-13a (10·7 kb); n, E1-14a (12·8 kb); o, E1-7b (2·8 kb); p, E1-6b (3·0 kb); q, E1-5b (5·0 kb); r, E1-4b (5·5 kb); s, E1-3b (7·0 kb); t, E1-2b (8·5 kb); u, E1-1b (9·2 kb); v, EV-2a (4·9 kb); w, EV-1a (7·3 kb); x, EV-1b (4·9 kb); y, Hd-4a (4·1 kb); z, Hd-3a (4·4 kb); aa, Hd-2a (6·0 kb); bb, Hd-1a (7·9 kb); cc, Hd-2b (6·0 kb); dd, Hd-1b (5·4 kb).

Southern bolts were similar in intensity, suggesting that they each contained most or all of the probe sequence. Among the bands we mapped, the only bands of less than normal intensity were E1-1a, E1-13a, E1-14a, and Hd-1a. In addition, occasional blots showed other faint bands. These bands may represent sequences containing only part of the probe region or sequences with considerable divergence from the probe sequence. We were not able to map these faint bands because they did not consistently appear on the autoradiograms.

Most of the mapping was done the *Eco*R I, which gives a pattern of clearly separated bands for each line. With this enzyme, line CM37 showed 10 clear bands, T232 showed 8 bands, Tx303 showed 5 bands, and CO159 showed 4 bands. However, not all the bands could be mapped, as lines T232 and CM37 had 2 bands of common size, at 2·1 and 6·4 kb, and lines Tx303 and CO159 had a common band 2·1 kb. In addition, all four lines had bands appearing at high molecular weight positions. These large bands could not be reliably scored. A total of 21 bands, the E1 series in Table 2, were mapped using *Eco*R I.

Because we were unable to map all the *Ac*-like sequences using *Eco*R I, we did further studies with *Hind* III and *Eco*R V. As can be seen in Fig. 1, these enzymes were not as successful at creating well separated bands, but we were able to map an additional 6 bands using *Hind* III (the Hd series) and 3 bands using *Eco*R V (the EV series).

Of the enzymes chosen, *Eco*R V does not cut within the standard *Ac* element, *Eco*R I cuts once in *Ac*, but not in the probed region, and *Hind* III cuts at two sites flanking the probed region within *Ac*. Thus, each standard *Ac* element should give a unique band when digested with *Eco*R I or *Eco*R V, but a common 1·6 kb band when cut with *Hind* III. This band has been observed in all 4 RI progenitors; it is too small to be seen in Fig. 1. The bands seen in *Hind* III blots at other positions represent non-standard *Ac*-like

https://doi.org/10.1017/S0016672300025295 Published online by Cambridge University Press

Table 1. *Mapping data*

TXCM lines

Element	Band	Line of origin	Recombinant-inbred line number																						
			2	4	6	7	9	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	28	29
241	E1-1a	CM37	−	−	−	−	+	0	+	+	−	+	−	+	+	−	−	−	+	+	+	−	−	−	−
242	E1-2a	CM37	−	−	−	+	−	0	+	+	+	−	+	−	−	+	−	−	−	+	−	+	−	−	+
243	E1-3a	T232	−	+	+	−	−	0	+	−	+	−	+	−	−	−	−	−	+	−	−	+	+	+	−
244	E1-4a	T232	+	+	+	−	+	0	−	−	−	+	−	+	+	−	+	+	+	−	+	−	+	+	−
245	E1-5a	CM37	+	−	−	+	−	0	−	−	−	−	+	+	−	−	−	+	−	−	−	+	−	−	+
246	E1-6a	CM37	+	−	−	−	−	0	+	−	−	−	−	+	−	+	−	−	−	−	+	+	+	−	+
247	E1-7a	CM37	+	+	−	−	−	0	−	−	−	−	+	−	−	+	−	−	+	+	−	+	−	−	−
248	E1-8a	T232	−	+	+	−	−	0	−	−	−	−	+	+	−	+	−	−	+	+	−	−	−	+	+
249	E1-9a	T232	−	−	+	+	+	0	+	+	+	+	−	+	+	−	+	+	+	−	+	−	+	+	+
250	E1-10a	CM37	+	+	+	−	−	0	−	+	+	−	−	+	+	+	+	+	+	+	+	−	−	+	−
251	E1-11a	T232	−	−	−	+	−	0	−	−	−	+	−	−	+	−	+	+	+	−	+	−	+	+	−
252	E1-12a	T232	−	−	−	−	+	0	+	+	−	−	−	−	−	−	+	−	−	+	−	+	+	−	+
253	E1-13a	CM37	+	−	+	+	−	0	−	+	+	+	+	−	+	−	+	+	+	−	+	−	−	−	+
322	Hd-2a	T232	+	+	+	−	+	0	−	−	−	+	−	−	+	−	+	+	+	−	+	−	+	+	−
326	EV-1a	CM37	+	+	−	−	−	0	−	−	−	−	+	−	−	+	−	−	+	+	−	+	−	−	−

COXTx lines

Element	Band	Line	Recombinant-inbred line number																						
			58	59	60	61	62	63	64	66	67	68	69	71	72	73	75	76	77	78	80	81	82	83	84
325	Hd-1b	CO159	−	−	+	−	0	−	+	−	+	−	0	−	−	−	+	−	+	−	−	0	+	−	−
328	EV-1b	CO159	+	−	+	+	0	+	+	+	+	+	0	−	−	−	+	+	+	+	+	−	−	−	−
336	EI-1b	CO159	−	−	+	−	0	−	+	−	+	+	0	−	−	−	+	−	+	−	−	0	+	−	−
337	EI-2b	Tx303	+	+	−	+	0	+	−	+	−	+	0	+	+	+	−	+	−	+	+	0	−	+	+
338	EI-3b	Tx303	−	−	−	+	0	+	−	+	+	−	0	−	+	+	+	+	+	+	−	0	−	−	+
339	EI-4b	CO159	+	−	+	+	0	+	+	+	+	−	0	−	−	−	+	+	+	+	−	0	+	−	+
340	EI-5b	CO159	−	+	+	−	0	+	−	+	−	+	0	−	+	−	+	+	+	−	+	0	+	−	+
341	EI-6b	Tx303	+	+	−	+	0	−	+	+	+	−	0	+	+	+	+	0	+	0	−	0	+	+	+
342	EI-7b	Tx303	+	+	+	+	0	+	+	+	−	−	0	+	+	+	+	−	−	+	+	0	−	+	+

A '+' indicates the presence of a band, a '−' indicates the absence of a band, and a '0' indicates no data.

sequences. These sequences might represent defective *Ac* elements, or *Ds* elements of the *Ac* deletion-derivative type (Fedoroff, Wessler & Shure, 1983).

For genetic mapping, 48 RI lines from the TXCM cross and 41 lines from the COXTx cross were examined. It was assumed that, for each *Ac* element, one allele was the presence of the band and the other allele was the absence of that band. No attempt was made to distinguish heterozygotes from homozygotes by relative band intensity. Also, no attempt was made to infer allelism between bands at different positions until the data were analyzed. Noting the presence or absence of each band in each RI line allowed us to map their chromosomal locations by comparison with the database of Burr *et al.* (1988).

The 30 bands on Southern blots could be resolved into 24 independent elements (Table 1), defined as sequences mapping to a unique position in the genome of its parental line. Several elements (*237, 241, 246, 247,* and *248*) were apparently mapped with two (or three) different enzymes. That is, a band mapped with one enzyme had the same distribution among the RI lines as another band mapped with a different enzyme. Thus, both bands were probably derived from the same *Ac*-like element. There is also a case in which two *Eco*R I bands, E1-6a and E1-14a, had identical distributions. These may represent tightly linked elements, or they may represent two pieces of the same element which happens to have an *Eco*R I site within the probed region. For the arguments and calculations to be presented below, we have assumed that when two bands from the same line have identical distributions, they are derived from a single *Ac*-like element, element *246* in this case.

(ii) *Allelism*

The recombinant-inbred technique is very good for rapid mapping to a region of a chromosome, but it is not capable of fine-scale mapping, because there are only a limited number of potential recombinants for any particular region. Map distances can only be

https://doi.org/10.1017/S0016672300025295 Published online by Cambridge University Press

Table 1 (*cont.*)

Element	Recombinant-inbred line number																							
	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	51	53	54	55	56	57
241	–	–	–	–	–	+	–	+	–	–	+	–	–	+	–	+	+	+	–	–	+	+	+	+	–
242	+	–	–	+	–	+	+	+	–	+	+	–	–	+	+	+	–	–	–	–	+	+	–	+	–
243	+	+	+	+	+	–	+	+	+	+	–	+	+	+	–	+	+	–	+	–	+	–	–	+	–
244	–	+	+	–	+	–	–	–	+	–	–	+	+	–	–	–	+	+	+	+	–	–	+	–	+
245	–	–	–	+	+	+	+	–	+	+	+	+	+	–	+	+	–	+	+	+	+	–	+	–	+
246	–	–	–	+	–	–	+	–	+	–	+	+	–	–	+	+	–	+	+	+	+	–	+	–	+
247	+	–	–	–	–	+	–	+	+	+	+	+	–	+	+	+	–	+	–	+	–	–	–	+	–
248	+	–	–	–	+	+	+	–	+	+	+	–	+	+	–	–	–	+	–	+	+	–	+	+	+
249	–	+	+	+	+	–	+	–	–	–	–	–	+	–	–	–	+	–	+	–	+	+	+	–	+
250	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	–	–	+	–	+	–	+	–	–	+
251	–	+	+	–	+	–	–	–	+	–	–	–	+	–	–	–	+	+	+	+	–	–	+	–	+
252	–	–	+	+	–	+	+	–	+	–	–	–	+	+	+	+	+	–	+	–	+	–	+	+	–
253	+	–	–	–	–	+	–	–	–	+	–	–	+	+	+	–	–	–	+	+	–	–	–	–	–
322	0	+	+	–	+	–	–	–	+	–	–	–	+	–	–	–	+	+	+	+	–	–	+	0	+
326	+	0	–	–	–	+	+	+	+	+	+	+	–	+	+	+	–	+	–	+	–	–	–	+	–

Element	Recombinant-inbred line number																	
	85	86	88	87	89	90	91	92	93	94	95	96	97	98	99	100	102	103
325	–	+	–	+	+	+	+	+	+	–	+	+	+	–	–	+	+	0
328	+	–	+	0	–	+	+	0	0	–	–	+	+	–	+	+	+	+
336	–	+	0	+	+	+	+	+	+	–	+	+	+	–	–	+	+	0
337	+	–	0	–	–	–	–	–	–	+	–	–	–	+	+	–	–	0
338	+	+	0	+	+	–	–	–	–	+	–	+	–	–	+	–	–	0
339	–	–	–	–	–	+	–	–	+	–	–	+	+	–	+	+	+	0
340	+	+	–	+	–	–	–	–	–	–	+	–	–	–	+	+	+	0
341	+	–	+	+	0	–	+	0	–	–	–	+	+	+	+	–	+	0
342	+	+	+	–	+	–	+	–	–	+	–	–	+	+	+	–	+	0

approximated, and it is impossible to determine true allelism between bands in different lines. At best, two bands from different parents can be shown to have identical distributions among the RI lines examined. This situation was found with 2 pairs of elements coming from opposite parents in RI crosses. Elements *242* and *244*, from CM37 and T232 respectively, have identical distributions among the TXCM lines, and we have assumed that they are allelic. Similarly, elements *325*, from CO159, and *337*, from Tx303, are considered to be allelic because they have identical allelic distributions in the COXTx lines. Elements *247* and *249* from CM37 and T232 had identical distributions except in recombinant-inbred line 22, which had both bands instead of one or the other. This RI line could be heterozygous for the region containing these elements, so these two elements might be allelic. Burr *et al.* (1988) found that 7·5% of the loci they mapped in the RI crosses were heterozygous.

There are three pairs of elements that map close to one another but which have been separated by recombination in at least one recombinant-inbred line. Elements *244* and *322* from T232 differ in lines 17 and 42, which contain element *244* but lack *322* (Fig. 2*a*). Elements *247* and *326* from CM37 differ in line 37, which carries *326* but not *247* (Fig. 2*b*). Elements *325* and *336* from CO159 differ in line 68, which contains *336* but not *325* (Fig. 2*c*). All other pairs of elements differed in at least 10 recombinant-inbred lines.

For bands from lines in different RI crosses, the closest approach to allelism is mapping to approximately the same location. Two cases of this phenomenon were found at locations away from chromosome 4. Elements *241* and *341* from lines CM37 and Tx303 were found in similar positions near locus *NPI421a* on chromosome 2. That is, the allelic distributions of these elements were quite similar to the distribution of *NPI421a*, mapped by Burr *et al.* (1988). For the analysis of element distribution presented below, these elements were considered

https://doi.org/10.1017/S0016672300025295 Published online by Cambridge University Press

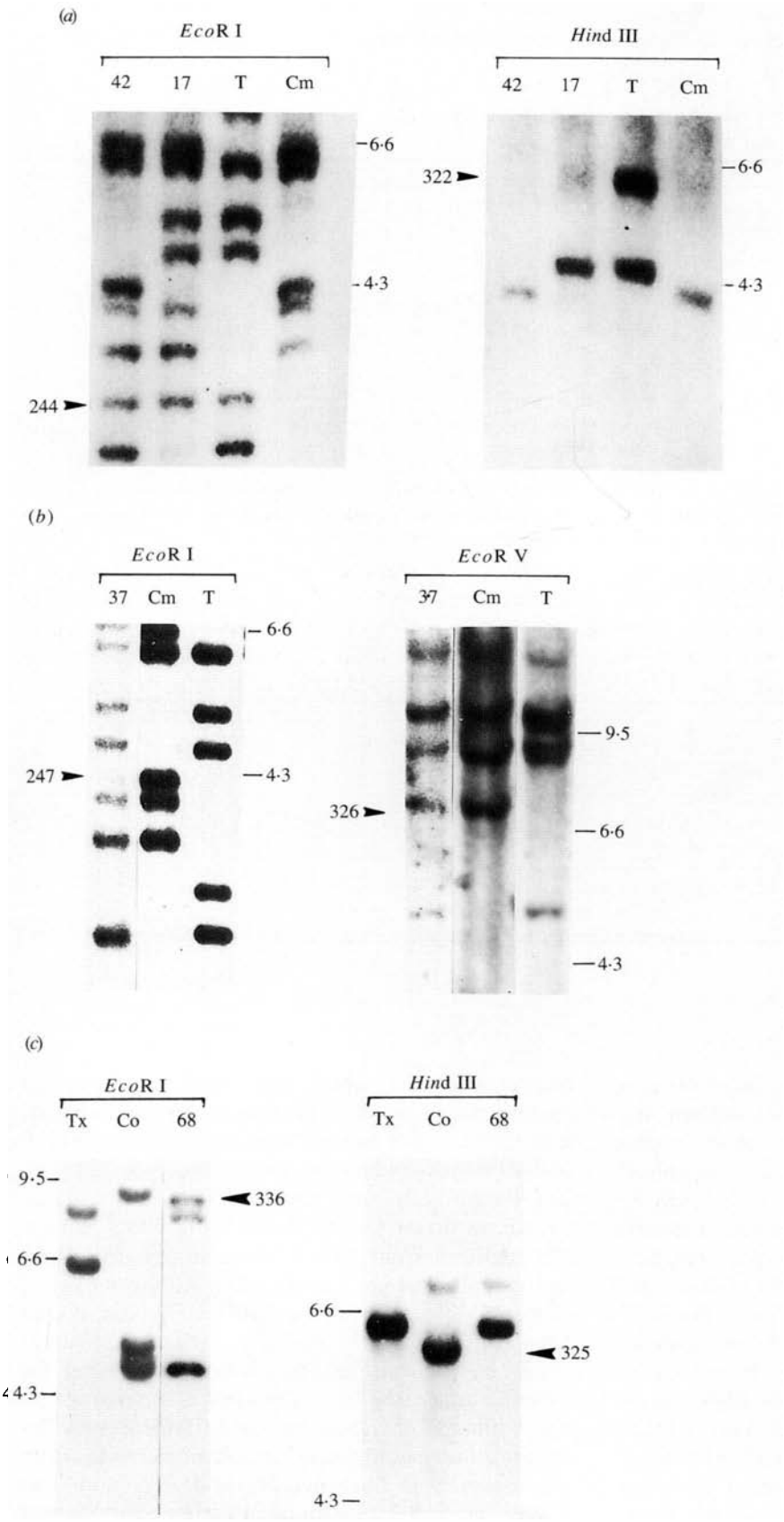

Fig. 2. Non-allelism between *Ac*-like sequences. (*a*) In T232, element *244* is not allelic to element *322* because *244* is present in recombinant-inbred lines 17 and 42 while *322* is absent in these line. (*b*) In CM37, element *247* is not allelic to element *326* because recombinant-inbred line 37 carries *326* but not *247*. (*c*) In CO159, elements *325* and *336* are not allelic because recombinant-inbred line 68 contains *336* but not *325*.

https://doi.org/10.1017/S0016672300025295 Published online by Cambridge University Press

allelic. Similarly, elements *250* and *338* from T232 and Tx303 mapped to similar positions near locus *1·556* on chromosome 1. However, element *252*, also found on chromosome 1 in CM37, differs from *250* in 11 of the 48 recombinant-inbred lines, indicating a loose linkage. Thus, 3 elements have been mapped near one another on chromosome 1, but only 2 separate sites have been demonstrated.

The elements on chromosome 4 can be divided into 4 groups. Group I, near *Adh2*, contains elements *249, 247, 326* and *339*. This cluster must contain at least two distinct sites, because *247* and *326* are not allelic. The average distance between the group I elements (as derived from the number of differences among the RI lines) is 2·2 cM. Group II, near *15·27*, contains elements *244, 322, 242, 325*, and *337*. This cluster must also contain at least 2 sites, because both *244* and *322*, and *325* and *336*, are not allelic. The average distance between the two distinguishable sites in group II is 2·2 cM. Group III, near *7·20L*, contains elements *251* and *342*. This cluster may consist of only a single site. Element *328* mapped to a unique position near *AASC94*. The large number of elements mapping to chromosome 4 and the crude nature of mapping with recombinant-inbreds make conjectures about allelism in this region difficult. If it is assumed that every chromosome 4 element found in clusters I, II or III in T232 or CM237 is allelic to an element in Tx303 or CO159, there would be a minimum of 6 independent sites containing *Ac*-like elements on this chromosome. In summary, our data show that the four inbred maize lines contain at least 24 *Ac*-like sequences among them, located at a minimum of 15 separable sites (Table 2).

(iii) *Distribution*

The *Ac*-like sequences can be divided into two groups, those located on chromosome 4 and those located elsewhere. Of the 24 mapped elements, 13 are on chromosome 4, and of the 15 definitely different sites, 6 are on chromosome 4. All four lines contain at least two chromosome 4 elements. In contrast, the elements off chromosome 4 are widely scattered, and no chromosome is represented by even one element in all four lines.

The *Ac*-like sequences represent a small number of objects distributed among a very large number of potential sites. Thus, if the locations of the *Ac*-like sequences are random and independent of one another, they should fit a Poisson distribution. To test for goodness-of-fit to a Poisson distribution, we have

Table 2. *Genomic positions of* Ac*-like sequences*

Line	Element	Band(s)	Chromosome	Nearest locus	Possible alleles
T232	243	EI-3a	6	NP1223	
	244	EI-4a	4-II	15·27	242 (CM37)
	248	EI-8a, Hd-3a	10	NPI285	
	249	EI-9a	4-I	Adh2	247 (CM37)
	250	EI-12a	1	1·556	338 (Tx303)
	251	EI-11a	4-III	7·20L	342 (Tx303)
	322	Hd-2a	4-II	15·27	
CM37	241	EI-1a, Hd-1a EV-2a	2	NPI421A	341 (Tx303)
	242	EI-2a	4-II	15·27	244 (T232)
	245	EI-5a	2	17·03+	
	246	EI-6a, EI-14a	2	17·03	
	247	EI-7a, Hd-4a	4-I	Adh2	249 (T232)
	252	EI-10a	1	5·59	
	253	EI-13a	7	8·37	
	326	EV-1a	4-I	Adh2	
CO159	325	Hd-1b	4-II	15·27	337 (Tx303)
	328	EV-1b	4	AASC94	
	336	EI-1b	4-II	15·27	
	339	EI-4b	4-I	Adh2	
	340	EI-5b	3	8·35	
Tx303	337	EI-2b, Hd-2b	4-II	15·27	325 (CO159)
	338	EI-3b	1	1·556	250 (T232)
	341	EI-6b	2	NPI421A	241 (CM37)
	342	EI-7b	4-III	7·20L	251 (T232)

https://doi.org/10.1017/S0016672300025295 Published online by Cambridge University Press

counted all the elements on a chromosome and determined how many chromosomes contained 0, 1, 2, ..., n elements. The observed data were compared to the expected distribution using the χ_2 test (Zar, 1984). No attempt was made to correct for different chromosome lengths. Three of the four inbred lines, T232, CM37, and CO159, show a strongly non-random distribution of *Ac*-like sequences. The probability of the observed chromosomal distributions of *Ac*-like sequences occurring by chance was less than 0·025 for any of these three lines. That is, these lines have an unexpectedly large number of elements on chromosome 4. Tx303 does not have a statistically significant non-random distribution. However, two of the four mapped elements in this line are on chromosome 4, and this line cannot be considered a counter-example to the other three lines. When the four lines are considered together, the minimum estimate of 15 sites (which may underestimate the actual number of chromosome 4 sites), gives a strong non-random distribution ($P < 0{\cdot}001$). If there are more than 6 sites on chromosome 4, the distribution becomes even more non-random. Thus, we have statistically shown that the *Ac*-like sequences in several inbred maize lines are not randomly distributed.

(iv) *Defective* Acs

The elements *241, 248, 322, 325*, and *337* were mapped using polymorphisms generated by *Hin*d III. Since this enzyme cuts the standard *Ac* element at positions flanking the probed region, these 5 elements are necessarily non-standard. They may represent *Ds* elements generated by internal deletions of *Ac*. However, their distribution is similar to that of the other elements: four are on chromosome 4 and one is elsewhere.

(v) *Full-length* Ac

Transposition of the *Ac*-like sequences in these lines could have occurred at any time in the past. However, while an explanation of the observed distribution does not require an intact *Ac* element in the present-day lines, it was of interest to determine whether an intact *Ac* sequence exists in any of these lines. To approach this question, we digested DNA from the four lines with *Acc* I, or *Dra* I plus *Bam*H I. Each of these combinations should yield a large internal fragment of an intact *Ac* element (Pohlman, Fedoroff & Messing, 1984). Figure 3 shows that the *Ac* probe hybridizes to multiple bands of varying size in these blots. It therefore seems unlikely that many of the *Ac*-like sequences are intact *Ac*s. However, bands of approximately the correct size can be seen for all lines with both digests. It should also be noted that *Hin*d III gives an appropriate 1·6 kb band with all four lines. Thus it is quite possible that intact but methylated *Ac* elements exist in some or all of these lines. Definitive proof of this statement would require cloning and sequencing a large number of elements, a project beyond the scope of the present work.

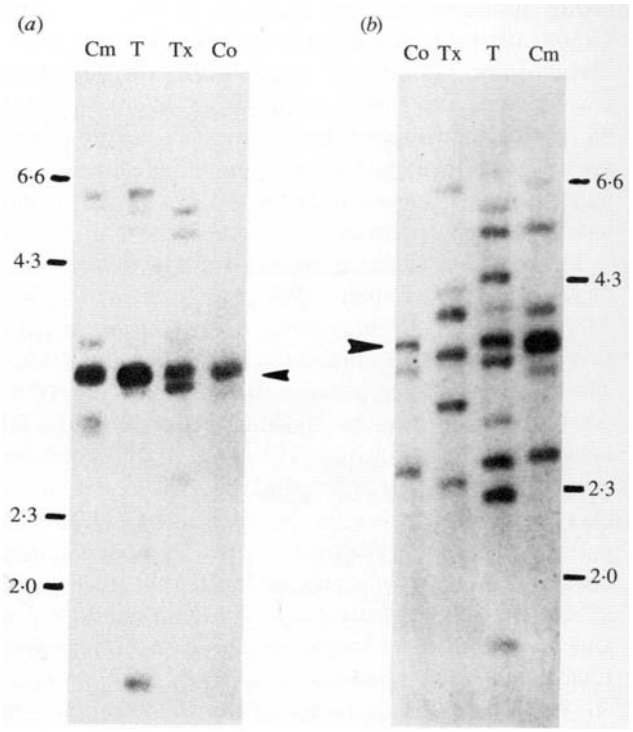

Fig. 3. Internal restriction fragments. (*a*) Digestion with *Acc* I. (*b*) Digestion with *Dra* I plus *Bam*H I. These enzymes produce internal fragments of 3388 bp and 3436 bp, respectively, from a standard *Ac* element. The recombinant-inbred progenitor lines digested with these enzymes contain bands of approximately the correct size (indicated by the arrows), as well as bands of other sizes.

4. Discussion

Assuming that *Ac*-like sequences are essentially *Ds* elements, defined by their ability to transpose under the influence of *Ac*, their distribution should depend on when and how often *Ac* elements have been activated. If transposition of *Ac*-like sequences had occurred only in the distant past, the positions of the elements would probably be identical in all lines. Genetic drift should insure that without transposition or strong selection for heterozygosity, the presence or absence of a sequence at any position will eventually become fixed (Montgomery, Charlesworth & Langley, 1987). The normal processes of genetic flux would alter the restriction sites around some of these loci, creating restriction fragment length polymorphisms that would allow the mapping of these sequences. Completely immobile transposable elements should act like normal gene loci.

Conversely, if transposition were very common in present-day maize, *Ac*-like sequences would not have identical locations in different lines, especially in the rather distantly related recombinant-inbred parents (Stuber & Edwards, 1986). Another corollary of

https://doi.org/10.1017/S0016672300025295 Published online by Cambridge University Press

frequent transposition, either in the present or in the past, is a random distribution of *Ac*-like sequences. Even though *Ac* usually moves to a linked location (Greenblatt, 1984), every jump to a new chromosome would result in a cluster of *Ac*s there. Eventually, all of the chromosomes would be infected, and, among the members of a large population, a large fraction of the potential sites would be occupied. Random drift and selection would then determine which elements survived.

Neither of the above extreme expectations were met. The *Ac*-like sequences were found at different positions in different lines. However, some pairs of lines had apparently allelic elements. These facts are hard to reconcile with either a no transposition model or a frequent transposition model. Also, the distribution of the elements is quite non-random, with every line having several copies on chromosome 4. This distribution is incompatible with many transposition events. However, the distribution of these sequences fits what would be expected from a limited number of transpositions starting with an initial element on chromosome 4.

Unlike *Drosophila* transposable elements, which transpose to random locations (Montgomery & Langley, 1983; Montgomery, Charlesworth & Langley, 1987), *Ac* usually transposes to a position linked to its original location. Preferential transposition to linked sites has been demonstrated for two independent *Ac* elements at two different sites, P^{vv} and *bz-m2*, and probably is true for all *Ac* elements (Greenblatt, 1984; Chen, Greenblatt & Dellaporta, 1987; Dooner & Belachew, 1989). The maize transposable element *En*, which has many similarities to *Ac*, was also shown to transpose to linked sites in approximately 25% of the cases studied (Peterson, 1970). The elements on chromosome 4 are linked to one another: element *328* is 21 cM from *339* in cluster I, the cluster I elements are an average of 24 cM from the cluster II elements, and the cluster II elements are an average of 19 cM from the cluster III elements. The distances between chromosome 4 clusters are similar to the 34 cM estimated as the average distance *Ac* transposes away from P^{vv} (Chen, Greenblatt & Dellaporta, 1987; Greenblatt, 1984). Thus, the distribution of *Ac*-like sequences in these lines could be modeled as an initial series of transpositions away from an original element located on chromosome 4, followed by segregation of the elements into different lines.

An *Ac*-like sequence that has transposed to a site on a different chromosome will probably transpose to further sites on that chromosome. Thus, secondary clusters of *Ac*-like sequences might be expected away from chromosome 4. We found two groups of linked elements on other chromosomes, which might represent secondary clusters of *Ac*-like sequences. On chromosome 1, elements *250, 252*, and *338* are all found near the *1·556* locus. Elements *250* and *252* (from T232 and CM37) are 15 cM apart. Element *338* is from line Tx303 and cannot be directly mapped relative to *250* and *252*, but its position relative to *1·556* suggests that allelism with *250* is more likely than with *252*. On chromosome 2, *246* and *245* are both near *17·03*, 15 cM apart. Elements *241* and *341* are on the opposite end of chromosome 2 from *246* and *245*, and probably represent an independent transposition onto this chromosome. Both of these regions contain fewer elements that are found on chromosome 4, suggesting a more recent origin for the secondary clusters. The presence of this apparent secondary clustering is further evidence that *Ac*-like sequences have transposed a limited number of times by a mechanism similar to that used by *Ac*.

Various copy number control mechanisms for transposable elements have been proposed, including selection against deleterious insertions, aneuploidy resulting from ectopic recombination between transposable elements at different locations, and explicit copy number control mechanisms such as are found with bacterial plasmids (Charlesworth & Charlesworth, 1983; Montgomery, Charlesworth & Langley, 1987). Underlying all of these mechanisms is the assumption that there is a steady-state number of elements in the genome. This assumption has not been proven for *Ac*-like sequences. Greenblatt (1966) has shown that the transposition of *Ac* produces an average of 1·44 *Ac* elements after each transposition event. Whether *Ac* is duplicated or not depends on whether it transposes ahead of or behind the replication fork. If *Ac* has been transposing at a low rate since its inception, only 5 or 6 transpositions per element would be necessary to generate the observed 5–10 copies per genome. Thus, it is possible that the number of *Ac*-like sequences observed is a historical accident and that no strong mechanism is currently controlling its numbers in lines which are genetically non-*Ac*.

It is interesting to note that no *Ac* sequences were found on chromosome 9, where much of McClintock's *Ac* work was done. Three well-studied *Ac* insertions, *bz-m2, wx-m7*, and *wx-m9* are located on this chromosome. Conversely, we are not aware of any *Ac* insertional mutants located on chromosome 4, despite the presence of the easily scored *C2* gene. There is a *Ds* insertion at *C2, c2-m*, but compared to other loci conditioning aleurone color, *C2* is not a common target for *Ac* or *Ds* (Fincham & Sastry, 1974).

The enzyme *Hin*d III generates internal fragments from standard *Ac* elements. Thus, the 5 *Ac*-like elements mapped by length polymorphisms generated by this enzyme represent non-standard *Ac*s. The simplest explanation for these sequences is that they are deletion derivatives of *Ac*, probably *Ds* elements of the *Ds6* type (Fedoroff, Wessler & Shure, 1983). The distribution of these elements is similar to that of the other *Ac*-like sequences. This result suggests that the occasional activation of *Ac* also results in the

trans-activation of *Ds* transposition. It has been shown that a hypermethylated and inactive *Ac* element can be induced to transpose by the introduction of an active *Ac* (McClintock, 1964, 1965; Schwartz & Dennis, 1986; Chomet, Wessler & Dellaporta, 1987). Thus it is likely that only a single demethylation event, the activation of an *Ac*, is necessary to induce transposition of *Ds* elements, which would result in their observed distribution.

References

Barclay, P. C. & Brink, R. A. (1954). The relation between Modulator and Activator in maize. *Proceedings of the National Academy of Science USA* **40**, 1118–1126.

Bennetzen, J. L. (1987). Covalent DNA modification and the regulation of Mutator element transposition in maize. *Molecular and General Genetics* **208**, 45–51.

Burr, B., Archer, K. & Burr, F. A. (1987). Activation of *Spm* with a chemical mutagen. In *Plant Transposable Elements* (ed. O. Nelson). New York: Plenum Press.

Burr, B., Burr, F. A., Thompson, K. H., Albertson, M. C. & Stuber, C. W. (1988). Gene mapping with recombinant inbreds in maize. *Genetics* **118**, 519–526.

Chandler, V. & Walbot, V. (1986). DNA modification of a maize transposable element correlates with loss of activity. *Proceedings of the National Academy of Sciences USA* **83**, 1767–1771.

Chandler, V. L., Talbert, L. E., Raymond, F. (1988). Sequence, genomic distribution and DNA modification of a *Mu1* element from non-Mutator maize stocks. *Genetics* **119**, 951–958.

Charlesworth, B. & Charlesworth, D. (1983). The population dynamics of transposable elements. *Genetical Research* **42**, 1–27.

Chen, J., Greenblatt, I. M. & Dellaporta, S. L. (1987). Transposition of *Ac* from the *P* locus of maize into unreplicated chromosomal sites. *Genetics* **117**, 109–116.

Chomet, P. S., Wessler, S. & Dellaporta, S. L. (1987). Inactivation of the maize transposable element *Activator* (*Ac*) is associated with its DNA modification. *EMBO Journal* **6**, 295–302.

Doerschug, E. B. (1973). Studies of Dotted, a regulatory element in maize. I. Induction of Dotted by chromatid breaks. *Theoretical and Applied Genetics* **43**, 182–189.

Dooner, H. K. & Belachew, A. (1989). Transposition pattern of the maize element *Ac* from the *bz-m2(Ac)* allele. *Genetics* **122**, 447–457.

Edwards, M. D., Stuber, C. W. & Wendel, J. F. (1987). Molecular marker facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics* **116**, 113–125.

Fedoroff, N., Wessler, S. & Shure, M. (1983). Isolation of the transposable maize controlling elements *Ac* and *Ds*. *Cell* **35**, 235–242.

Feinberg, A. P. & Vogelstein, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132**, 6–13.

Fincham, J. R. S. & Sastry, G. R. K. (1974). Controlling elements in maize. *Annual Review of Genetics* **8**, 15–50.

Greenblatt, I. M. (1966). Transposition and replication of *Modulator* in maize. *Genetics* **53**, 361–369.

Greenblatt, I. M. (1968). The mechanism of *Modulator* transposition in maize. *Genetics* **58**, 585–597.

Greenblatt, I. M. (1974). Movement of *Modulator* in maize: a test of an hypothesis. *Genetics* **77**, 671–678.

Greenblatt, I. M. (1984). A chromosomal replication pattern deduced from pericarp phenotypes resulting from movements of the transposable element, *Modulator*, in maize. *Genetics* **108**, 471–485.

Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor: Cold Spring Habor Press.

McClintock, B. (1947). Cytogenetic studies of maize and *Neurospora*. *Carnegie Institution of Washington Yearbook* **46**, 146–151.

McClintock, B. (1951). Chromosome organization and gene expression. *Cold Spring Harbor Symposium on Quantitative Biology* **16**, 13–47.

McClintock, B. (1964). Aspects of gene regulation in maize. *Carnegie Institution of Washington Yearbook* **63**, 592–602.

McClintock, B. (1965). Components of action of the regulators *Spm* and *Ac*. *Carnegie Institution of Washington Yearbook* **64**, 527–536.

McClintock, B. (1978). Mechanisms that rapidly reorganize the genome. *Stadler Genetics Symposium* **10**, 25–48.

McClintock, B. (1984). The significance of responses of the genome to challenge. *Science* **226**, 792–801.

Montgomery, E. A. & Langley, C. H. (1983). Transposable elements in Mendelian populations. II. Distribution of three *copia*-like elements in a natural population of *Drosophila melanogaster*. *Genetics* **104**, 473–483.

Montgomery, E., Charlesworth, B. & Langley, C. H. (1987). A test for the role of natural selection in the stabilization of transposable element copy number in a population of *Drosophila melanogaster*. *Genetical Research* **49**, 31–41.

Mottinger, J. P., Johns, M. A. & Freeling, M. (1984). Mutations of the *Adh1* gene in maize following infection with barley stripe mosaic virus. *Molecular and General Genetics* **195**, 367–369.

Peschke, V., Phillips, R. L. & Gengenbach, B. G. (1987). Discovery of transposable element activity among progeny of tissue culture-derived maize plants. *Science* **238**, 804–806.

Peterson, P. A. (1970). The *En* mutable system in maize. III. Transposition associated with mutational events. *Theoretical and Applied Genetics* **40**, 367–377.

Peterson, P. A. & Salamini, F. (1986). A search for active mobile elements in Iowa stiff stalk synthetic maize population and some derivatives. *Maydica* **31**, 163–172.

Pohlman, R., Fedoroff, N. V. & Messing, J. (1984). The nucleotide sequence of the maize controlling element *Activator*. *Cell* **37**, 635–643.

Rhoades, M. M. & Dempsey, E. (1982). The induction of mutable systems in plants with the high-loss mechanism. *Maize Genetics Coop Newsletter* **56**, 21–26.

Saghai-Maroof, M. A., Soiman, K. M., Jorgenson, R. A. & Allard, R. W. (1984). Ribosomal DNA spacer-length polymorphisms in barely: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences USA* **81**, 8014–8018.

Schwartz, D. & Dennis, E. (1986). Transposase activity of the *Ac* controlling element in maize is regulated by its degree of methylation. *Molecular and General Genetics* **205**, 476–482.

Southern, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503–517.

Sprague, G. F. & McKinney, H. H. (1966). Aberrant Ratio: an anomaly in maize associated with virus infection. *Genetics* **54**, 1287–1296.

Stuber, C. W. & Edwards, M. D. (1986). Genotype selection for improvement of quantitative traits using molecular marker loci. *Proceedings of the 41st annual Corn and Sorghum Research Conference*, pp. 70–83.

Walbot, V. (1986). Reactivation of somatic instability in an inactive Mutator stock by treatment of seed with gamma rays. *Maize Genetics Coop Newsletter* **60**, 124.

Zar, J. H. (1984). *Biostatistical Analysis*. Englewood Cliffs, New Jersey: Prentice-Hall.