THE *Mu* TRANSPOSABLE ELEMENTS OF MAIZE: EVIDENCE FOR TRANSPOSITION AND COPY NUMBER REGULATION DURING DEVELOPMENT

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Manuscript received April 12, 1985
Revised copy accepted September 23, 1985

ABSTRACT

The *Mu* transposon of maize exists in a highly mutagenic strain called Robertson's Mutator. Plants of this strain contain 10–50 copies of the *Mu* element, whereas most maize strains and other plants have none. When Mutator plants are crossed to plants of the inbred line 1S2P, which does not have copies of *Mu*, the progeny plants have approximately the same number of *Mu* sequences as did their Mutator parent. Approximately one-half of these copies have segregated from their parent and one-half have arisen by transposition and are integrated into new positions in the genome. This maintenance of copy number can be accounted for by an extremely high rate of transposition of the *Mu* elements (10–15 transpositions per gamete per generation). When Mutator plants are self-pollinated, the progeny double their *Mu* copy number in the first generation, but maintain a constant number of *Mu* sequences with subsequent self-pollinations. Transposition of *Mu* and the events that lead to copy number maintenance occur very late in the development of the germ cells but before fertilization. A larger version of the *Mu* element transposes but is not necessary for transposition of the *Mu* sequences. The progeny of crosses with a Mutator plant occasionally lack Mutator activity; these strains retain copies of the *Mu* element, but these elements no longer transpose.

IN 1978, ROBERTSON described a maize stock that generated 50–100 times the expected frequency of recessive mutations as assessed by scoring for kernel and seedling mutants in progeny of self-pollinated plants (ROBERTSON 1978). About 40% of these newly induced mutations were genetically unstable, suggesting that they were the result of insertions (GREEN 1977). ROBERTSON has characterized the mutator quality of this stock and named it Mutator (ROBERTSON 1978, 1980, 1981a,b). He showed that the mutagenic activity that defines Mutator stocks was transmitted as a dominant phenotype in crosses to non-Mutator stocks. When a Mutator plant was used as either the male or female parent approximately 90% of the progeny inherited Mutator activity (ROBERTSON 1978). Further, fate maps of mutant sectors in the ear suggested that new mutants are induced primarily late in plant development (ROBERTSON

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1980, 1981b). Thus, Mutator appears to be dissimilar to the bipartite controlling elements in maize described by McCLINTOCK (1950), RHOADES (1938), PETERSON (1960) and others, in which autonomous and nonautonomous components segregate in a Mendelian fashion as discrete genetic units (for reviews, see FEDOROFF 1983; FREELING 1984).

Molecular analysis of the Mutator system began with the selection of an unstable mutant allele of the *alcohol dehydrogenase-1* (*Adh1*) gene from a Mutator background (FREELING 1984; FREELING, CHENG and ALLEMAN 1982). This mutant and its progenitor were analyzed by genomic blots (STROMMER *et al.* 1982), cloned (BENNETZEN *et al.* 1984) and sequenced (BARKER *et al.* 1984; DENNIS *et al.* 1984). The mutant allele contains a 1.4 kilobase pairs (kbp) insertion, called *Mu1*. The sequence of *Mu1* is similar to other transposons in that it has inverted repeats 230 base pairs (bp) in length and is flanked by direct repeats of host DNA. In addition, *Mu1* has internal direct repeats of approximately 100 bp and also has symmetry about a central AT rich region. Three long, open reading frames of unknown function have been deduced from sequence analysis (BARKER *et al.* 1984).

DNA gel blot analyses (performed under stringent hybridization conditions using an internal *Mu1* fragment as a probe) show a unique correlation: Mutator stocks have between 10 and 50 intact and dispersed *Mu* sequences of two distinct size classes, whereas non-Mutator stocks have none (BENNETZEN 1984). This correlation suggests that the *Mu* element is involved in the Mutator phenomenon, but does not prove that it is solely responsible for Robertson's Mutator activity. There is evidence, however, that the *Mu* element is a major component of Mutator activity. First, all mutations that have been induced in Mutator lines and analyzed at the molecular level contain *Mu* insertions. These mutations include three other mutants of *Adh1* (JOHNS, ALLEMAN and FREELING 1983; J. N. STROMMER and M. A. JOHNS, unpublished results; C.-H. CHEN, K. OISHI and M. FREELING, unpublished results), a shrunken endosperm mutant allele, *sh1-mu9626*, (D. S. ROBERTSON, B. BURR and M. FREELING, personal communication) and a mutant in anthocyanin biosynthesis, *a1-Mum2* (O'REILLY *et al.* 1985). Second, a Mutator background does not act as a general activator of transposable elements; mutant alleles involving the *Ds/Ac*, *I/En*, *a1-m/Dt* and *r-cu/Fcu* systems do not become unstable in a Mutator background (ROBERTSON and MASCIA 1981).

Our studies pertain to the molecular basis of Robertson's Mutator activity. In this report we show that (1) *Mu1* elements transpose and demonstrate copy number maintenance; (2) *Mu* transposition occurs at an extremely high frequency before fertilization in germinal cells; (3) a larger 1.7-kbp version is an active element, but is not a "master"; and (4) plants that lose Mutator activity still have copies of *Mu*, but these elements no longer transpose.

MATERIALS AND METHODS

Stocks: The Mutator stock, DR74-2031-6 × 1028-8, was a gift of D. S. ROBERTSON (Iowa State University). In order to establish a Mutator stock with a specific genetic background, we crossed the Mutator stock to the inbred line 1S2P, followed by four backcrosses to this inbred. 1S2P is an inbred line from the FREELING lab; it does not

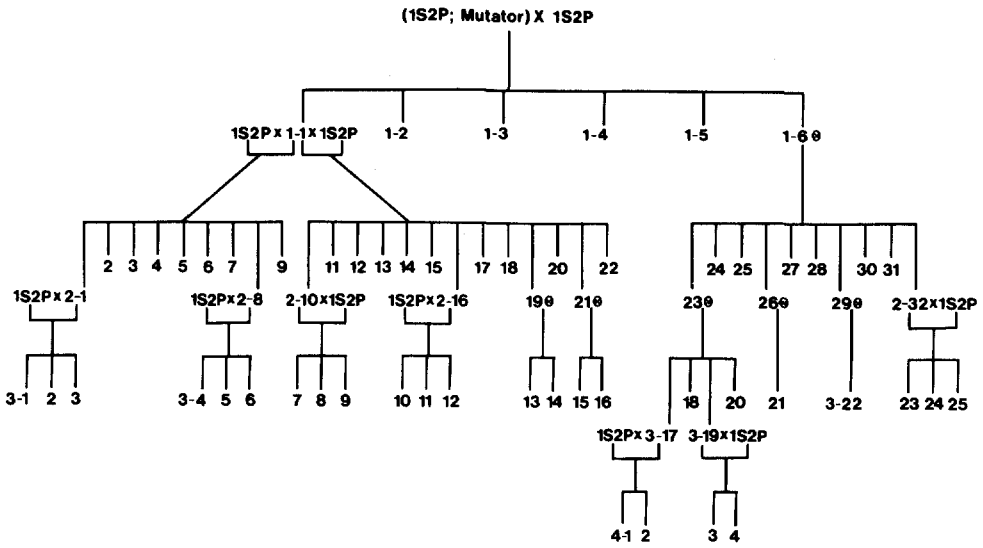


FIGURE 1.—Family tree of the 1S2P;Mutator lineage used in these experiments. Individuals are numbered with respect to the generation they occupy (1–4) and the position within their generation (from left to right). The 1S2P;Mutator stock was the result of five generations of crosses of Mutator from the initial stock DR 74-2031-6 × 1028-8 into the inbred line 1S2P. 1S2P has no detectable copies of *Mu* elements (Figure 3). The female parent is on the left in the crosses. ⊗ denotes a self-pollination.

show an increased frequency of new mutants relative to other inbred lines (M. FREELING, unpublished data). The result of this backcrossing was a line that was largely 1S2P in genetic background and retained Mutator activity. We designate this stock 1S2P;Mutator. The subsequent lineage of 1S2P;Mutator includes a number of self-pollinations and backcrosses to 1S2P (Figure 1). Mutator was also incorporated into another stock (d708a) by crossing it to 1S2P;Mutator and then backcrossing and self-pollinating. The d708a stock was obtained from M. G. NEUFFER (University of Missouri). Some stocks in which Mutator activity was lost were provided by ROBERTSON; these included DR77-2071-6N × 1070-8 and its descendants, which were outcrossed to non-*Mu* lines. In this paper, we use the term "Mutator" for plants of the Mutator genetic background that have the ability to produce new mutations at a high frequency. The term *Mu* refers to the genetic elements, *Mu1* and *Mu2*; these elements are associated with active Mutator stocks and with a number of mutations that have been induced in these stocks.

DNA isolation and DNA gel blot analysis: Mature maize leaves (5–10 g of tissue, wet weight) were quick frozen in liquid N_2 and were powdered with dry ice in an electric coffee grinder. DNA was isolated by the procedure of MURRAY and THOMPSON (1980).

Aliquots of DNA (15 μg) were digested with restriction enzyme for 3 hr using six units of enzyme per μg of DNA for the enzyme *Tth*111-I, or three units of enzyme per μg of DNA for other enzymes. Restriction enzymes were purchased from New England Biolabs, Inc.; buffer conditions for the enzymes were as suggested by the manufacturer. Gel electrophoresis through 0.8–1.0% agarose, DNA gel blotting and filter hybridization were performed as described (JOHNS, STROMMER and FREELING 1983). Gene Screen (New England Nuclear, Du Pont) was used as the blotting matrix.

Probes: The *Mu1* probe, pMuED2, was provided by E. DANIELL. The plasmid pMuED2 consists of a *Sau*3AI/*Hin*dIII fragment from the *Adh1-S3034* genomic clone (BENNETZEN *et al.* 1984) that was inserted into pBR322; it contains all of *Mu1* and 20

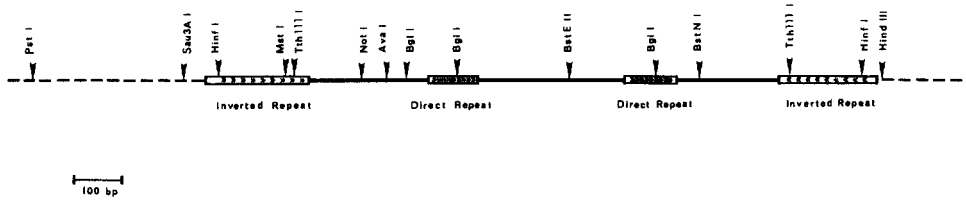


FIGURE 2.—Restriction map of a portion of the *Adh1-1S3034* genomic clone containing the *Mu1* transposable element of maize. *Mu1* was isolated from the unstable Mutator-induced mutant of the *Adh1* gene (*Adh1-1S3034*) (BENNETZEN *et al.* 1984). Position of restriction sites and direct and inverted repeats are based on the DNA sequence analysis of BARKER *et al.* (1984). The dashed line denotes the portion of the cloned DNA that is *Adh1* sequence.

bp of the surrounding *Adh1* sequence. Figure 2 is a restriction map of the *Mu1* element showing the region included by this *Mu1*-containing subclone. An *Adh1* unique sequence probe was also used for these experiments; this probe was a 1.5-kbp *Sac*II fragment gel purified from pB428 that includes the *Adh1-1S* allele (BENNETZEN *et al.* 1984).

Mu1-containing plasmid DNA or *Adh1* DNA was nick-translated to a specific activity of at least 10^8 cpm/μg DNA with α-^{32}P deoxynucleotides (RIGBY *et al.* 1977). The dried filters were hybridized in a solution containing Denhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 1.0 M NaCl, 0.05 M sodium phosphate, pH 7.4, 1 mM EDTA (ethylenediaminetetraacetic acid), 0.2% SDS (sodium lauryl sulfate), 0.15 mg/ml denatured salmon sperm DNA and 50 μg/ml polyadenylic acid. Hybridization was carried out at a temperature of 68° for 30–48 hr. Blots were washed at 65° for 2–3 hr with 0.2× SSC and 0.1% SDS. Autoradiography was carried out using Kodak XAR5 film with an intensifying screen. After the film was developed, a Transidyne General 2970 scanning densitometer was used in the quantification of band intensity. All exposures were in the linear range of the film.

RESULTS

Estimation of Mutator copy number: To estimate the number of *Mu* sequences in Mutator plants relative to the number in their progeny, it was first necessary to establish rigorous controls for the quantification of *Mu* copy number using DNA gel blot analysis.

Genomic DNA was digested with the restriction enzyme, *Tth*111-I, which cuts *Mu1* within the terminal inverted repeats to produce a fragment of 1.0 kbp. The large *Mu* element, *Mu2*, is also cut by enzymes that recognize sequences in the terminal repeats of *Mu1*; these enzymes (*Hin*fI and *Tth*111-I, for example) produce a *Mu2* restriction fragment that is consistently 0.4-kbp larger than the *Mu1* fragment (BENNETZEN 1984). DNA gel blot analysis of *Tth*111-I-digested genomic DNA, probed with pMuED2, produced two bands at 1.0 and 1.4 kbp accounting for all *Mu* elements in the genome (Figure 3, lanes 1–7). Because the entire *Mu1*-containing plasmid, pMuED2, was used as a hybridization probe, plasmid contamination and, therefore, the possibility of *Mu1*-plasmid contamination of our genomic DNA samples could be detected by the presence of additional (pBR322) bands on the DNA gel blots. Any lane that showed plasmid contamination was eliminated from the analyses.

Mu1-containing plasmid DNA, diluted to levels equivalent to various genomic copy numbers, was also run on each gel. An estimate of 6×10^9 bp

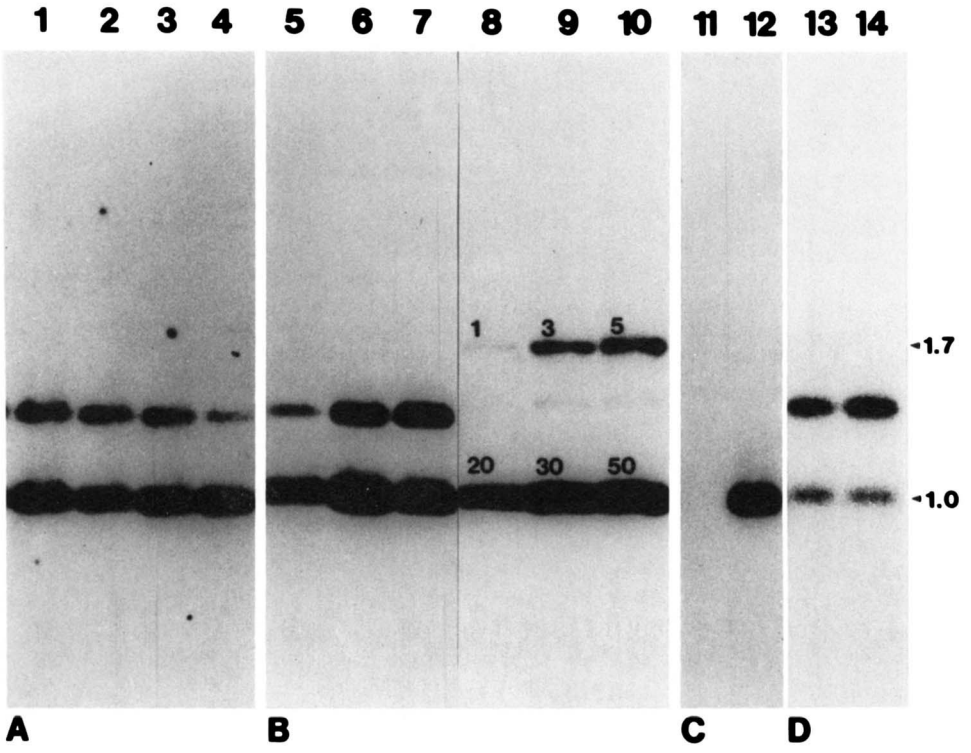


FIGURE 3.—*Mu* copy number following crosses and self-pollinations of Mutator plants. Maize DNA from mature leaves (15 μg/lane) was digested with the restriction enzyme *Tth*111-I. DNA gel blots of 0.8% agarose gels were probed with the nick-translated *Mu1*-containing plasmid, pMuEd2. These panels (A–D) were taken from four DNA gel blots. Figure 1 contains individual designation scheme. Panel A, lanes 1–4: Three generations of direct parent-to-offspring crosses of the 1S2P;Mutator stock to the non-*Mu* 1S2P. Lane 1 = individual 1-1; lane 2 = individual 2-1; lane 3 = individual 3-1; lane 4 = individual 1-6. Panel B, lanes 5-7: Three generations of direct parent-to-offspring *Mu* transmission by self-pollination of the 1S2P;Mutator stock. Lane 5 = individual 1-6; lane 6 = individual 2-23; lane 7 = individual 3-19. A and B have one individual in common, 1-6 (lanes 4 and 5). Lanes 8–10: Copy number controls of *Mu1*-containing plasmid DNA diluted to produce 1, 3, 5, 20, 30 and 50 genomic equivalents of *Mu1*. *Mu* copy number estimates for the individuals in panels A and B are individual 1-1 (23); individual 2-1 (27); individual 3-1 (25); individual 1-6 (18); individual 2-23 (40); and individual 3-19 (35). Panel C, lane 11: non-*Mu* control 1S2P. Lane 12: An example of an active Mutator line (HV698) with no *Mu2*. Panel D, lanes 13–14: Two sibling d708a;Mutator plants showing the high fraction of the total *Mu* sequences represented by *Mu2*. (Sizes of marker bands are shown in kbp).

was used for the size of the maize genome (H. J. PRICE, personal communication). These controls were used to provide an estimate of the total number of copies of *Mu1*-hybridizable sequences per genome for each plant. These copy number controls consisted of a *Mu1* subclone, digested under two restriction enzyme regimens. Plasmid was digested with *Tth*111-I to produce a *Mu1* fragment of 1.0 kbp, or with *Pst*I and *Hin*dIII to yield a *Mu1*-containing fragment of 1.7 kbp (see Figure 2). The *Tth*111-I and the *Pst*I/*Hin*dIII copy

number controls were normalized against each other on DNA gel blots. They were also compared with the genomic single copy *Mu* standards: the *Mu*-containing bands that appear on DNA gel blots when genomic DNA from a Mutator plant is digested with an enzyme that does not cut within either *Mu* element. In this case, fragments are distributed over a large range of molecular weights and represent single *Mu* elements at unique sites in the genome. Copy number standards were diluted to yield genomic equivalents of 1–50 copies of *Mu* per diploid genome; they were aliquotted for use on all DNA gel blots performed in these experiments. Non-Mutator 1S2P DNA that had been digested with the enzyme *Eco*RI was mixed with copy number standards to avoid blotting artifacts resulting from differences in DNA concentrations. DNA from 1S2P plants also served as a negative control for each DNA gel blot; no *Mu* sequences were detected in these plants (BENNETZEN 1984). Under high stringency wash conditions, 1S2P DNA showed no detectible hybridization to the *Mu1* probe (Figure 3, lane 11), but did to the *Adh1* probe. All blots were probed twice: first with the *Mu1* probe and then with the *Adh1* probe to control for variation in the amount of DNA loaded per lane.

Mutator copy number in outcrossed individuals: These experiments were performed in order to assess change in the number of *Mu* elements when a Mutator plant was crossed to a plant with no *Mu* elements, or when these plants were self-pollinated. Our study incorporated 35 individuals from the 1S2P;Mutator lineage in direct parent-to-offspring comparisons of Mutator plants and their progeny produced by backcrossing to the non-*Mu* inbred line 1S2P. Among these were five plants (individuals 1-1, 2-1, 2-8, 2-10 and 2-16; Figure 1) that were compared with 34 progeny by DNA gel blot analysis. In parent-to-offspring comparisons of copy number, analyses were always from the same DNA gel blot.

After densitometer tracings were performed for each individual and controls, an estimate of the *Mu* content of each individual was expressed as the number of densitometer deflection units caused by the *Mu1* band plus the number of densitometer deflection units caused by the *Mu2* band; this value was normalized for variation in the amount of DNA loaded in each lane using the percent of the *Adh1*-specific densitometer deflection for individuals on the same DNA gel blot (when it was probed with radiolabeled *Adh1*). Densitometer data could then be expressed as a copy number estimate by comparison with copy number standards, or as an arbitrary value of densitometer units. Because we were interested in how the *Mu* copy number changed in the progeny generation relative to the parental generation when a Mutator plant was crossed to a plant without *Mu* elements, copy number changes were expressed as a ratio of the densitometer units (progeny) divided by the densitometer units (parent). For the 22 progeny of individual 1-1, the ratio of *Mu* copies compared to this parent was 1.02 ± 0.10. For 29 parent/progeny comparisons, the ratio was 1.05 ± 0.12. We consistently found that the progeny of crosses involving Mutator plants by non-Mutator plants have approximately the same number of *Mu* copies as did their Mutator parent. Individual 2-16 and its three progeny were an exception and will be discussed in the section on Mutator

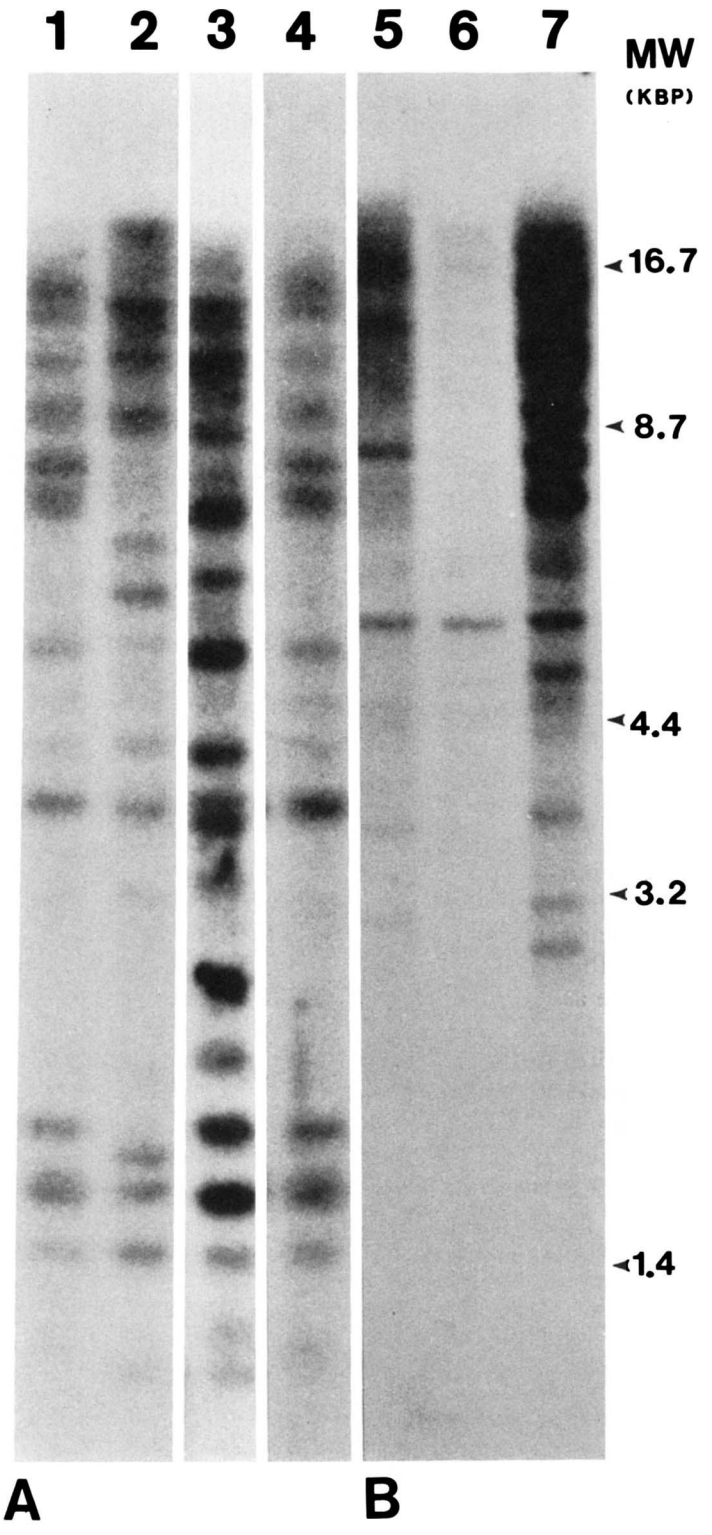
loss. The 1S2P;Mutator lineage maintained approximately 20–30 copies of *Mu* that include 20–30 copies of the 1.4-kbp *Mu1* element and 1–10 copies of the 1.7-kbp *Mu2* element. Lanes 1–4 of Figure 3 show representative DNA gel blot profiles of three generations in a direct parent-to-offspring lineage.

Mutator copy number after self-pollination: The results for *Mu* copy number following self-pollination of Mutator lines are strikingly different from those observed for crosses. DNA gel blots from individuals 1-6, 2-19 and 2-21 and their 14 progeny produced by self-pollination were performed, and densitometer tracings were compared. The densitometer deflection unit value for each progeny was expressed as a ratio compared to its parent. In all 14 parent-to-progeny comparisons, the progeny had 2.2 ± 0.21 times the *Mu* sequences as did their parents. In order to determine the effect of subsequent self-pollination on the *Mu* copy number, we compared plants 2-23, -26 and -29 with their six progeny. The second generation of self-pollination did not further increase the number of *Mu* sequences in the genome. The progeny had 0.95 ± 0.15 times the *Mu* sequences as did their parent. Lanes 5-7 of Figure 3 show representative DNA gel blot profiles of three generations of continuous self-pollination in a direct parent-to-offspring lineage.

Analysis of individual *Mu* elements by *SacI* digestion: When DNA from a Mutator plant is digested with a restriction enzyme that does not cut within either the 1.4-kbp or the 1.7-kbp *Mu* element, for example *Sac*I, and the resulting DNA gel blot is probed with pMuED2, a ladder of readily discernible bands appears. These bands are *Mu*-containing restriction fragments that represent *Mu* elements at unique genomic locations. We analyzed two Mutator plants (individuals 1-1 and 2-8) and 25 of their progeny by this method. Lanes 1–4 of Figure 4 show a representative DNA gel blot that compares a Mutator parent and its progeny after crosses with non-*Mu* 1S2P. When progeny were compared with their Mutator parent, an apparent conservation in band number was observed. Of these *Mu*-containing DNA fragments, approximately one-half were the same size as fragments in the parent, and one-half were at molecular weight positions not seen in the parent. This suggests transposition of *Mu* elements to new sites in the genome.

A large *Mu* element transposes but is not a "master": Transposition of the 1.7-kbp *Mu* element (*Mu2*) was shown by comparison of DNA gel blot profiles of 1S2P;Mutator and d708a;Mutator. The strain d708a;Mutator was generated by crossing 1S2P;Mutator to the non-*Mu* d708a stock, followed by backcrossing to 1S2P. The 1S2P;Mutator plants had 1–3 copies of *Mu2*, whereas d708a; Mutator had 15–20 copies of *Mu2*. The *Mu1* copy number in 1S2P;Mutator is approximately 20; only about 5 copies of *Mu1*, however, are in d708a; Mutator. The observed increase in *Mu2* copy number can only be explained by *Mu2* transposition. A DNA gel blot profile of DNA from two siblings of the d708a;Mutator stock is shown in Figure 3, lanes 13 and 14.

Transposition of *Mu1* in the absence of *Mu2* is shown by the presence of active Mutator stocks that have no copies of *Mu2*. Individual 2-32 has lost detectable copies of *Mu2* (not shown); copy number was maintained in crosses involving 2-32. In addition, two traits of active Mutator stocks, somatic leaf


1
2
3
4
5
6
7
MW
(KBP)
16.7
8.7
4.4
3.2
1.4
A
B

striping (FREELING 1984) and the induction of new mutants, occurred in this lineage. Copy number maintenance and mutagenesis in this stock indicate that *Mu2* is not necessary for Mutator activity or for transposition of *Mu1*. Lane 12 of Figure 3 shows a DNA gel blot profile of DNA from an active Mutator plant containing no *Mu2* elements.

***Mu* sequences in stocks that have lost Mutator activity:** ROBERTSON (1978) observed that, following crosses of Mutator by non-Mutator plants, approximately10% of the progeny do not inherit Mutator activity from their parent; these Mutator-loss plants do not regain Mutator activity following subsequent crosses to non-Mutator stocks. We analyzed several individuals that had lost Mutator activity. One of these stocks includes DR77-2071-6N × 1070-8 and eight generations of outcrosses to non-*Mu* stocks; others are individuals 2-4 and 2-16 (Figure 1) and their progeny. These stocks lose *Mu* copies at a rate of one-half each generation. DNA from these stocks was digested with a restriction enzyme that does not cut within the *Mu* elements. Individual 2-16 had 12 copies of *Mu*, approximately one-half of the number detected in its parent. This rate of loss is consistent with segregation of *Mu* elements in the absence of transposition. Figure 4, lanes 5–7 show DNA gel blots of DNA from three related plants in a lineage following Mutator loss.

DISCUSSION

These experimental results provide a molecular explanation for a set of characteristics observed for the Robertson's Mutator line of maize. These characteristics include an extremely high mutation frequency (ROBERTSON 1978, 1985), somatic leaf striping (FREELING 1984), chromosome breakage (M. ALLEMAN, unpublished results) and the loss of Mutator activity by 10% of outcrossed progeny (ROBERTSON 1978). All of these phenotypes can be explained by the behavior of transposable elements. One family of elements, the *Mu* transposons, has been identified; these are probably the only transposable elements active in Mutator lines. Our studies are directed toward the relationship between the *Mu* transposons and the Robertson's Mutator phenomenon.

The data presented above show that, in the 1S2P;Mutator lineage, approximately 10–15 new *Mu* insertions are detected per generation during sequential outcrossing of Mutator lines. This is equivalent to approximately one trans-

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FIGURE 4.—The distribution of *Mu* elements in Mutator plants and plants that have lost Mutator activity. Maize DNA from mature leaves (15 μg/lane) was digested with the restriction enzymes *Sac*I (lanes 1–4) or *Bam*HI (lanes 5–7). DNA gel blots of 0.8% agarose gels were probed with the nick-translated *Mu1*-containing plasmid, pMuED2. Lanes 1–4 compare the distribution of *Mu* sequences in *Sac*I-digested DNA of a Mutator plant and its progeny following outcrossing to non-*Mu* 1S2P. The Mutator plant, individual 1-1, was outcrossed as a male and as a female (see Figure 1). Lanes 1 and 4: Parent plant 1-1. Lanes 2 and 3: Progeny plants 2-10 and 2-11. Lanes 5–7 show DNA gel blot profiles of *Mu* elements in plants that have lost Mutator activity during outcrossing. Lane 5: Second generation of outcrossing Mutator loss line to the non-*Mu* hybrid B70. Lane 6: Fifth generation of outcrossing Mutator loss line to B70 and 1S2P. Lane 7: DR77-2071-6N × 1070-8 (Mutator activity loss plant). The individuals from which these DNAs were extracted do not share a parent-to-offspring relationship, but are related by lineage.

position event per *Mu* element per generation. The presence of newly transposed *Mu* elements is indicated by total copy number conservation and by the appearance of new *Mu*-containing restriction fragments in the progeny of outcrossed Mutator plants. We equate these new restriction fragments with transposition events for the following reason. Parental *Mu*-containing restriction fragments segregate in a Mendelian fashion to progeny. Segregation should decrease copy number by one-half each generation when crosses are made to non-*Mu* lines, such as 1S2P. Instead of the predicted reduction, copy number is maintained at 20–25 copies during sequential outcrossing to plants with no *Mu* elements. The observed *Mu* copy numbers and the stability of copy number from generation to generation may reflect the near-isogenic nature of the line used (1S2P;Mutator crossed to 1S2P without Mutator). Other Mutator lines have other *Mu* copy number values (BENNETZEN 1984).

We interpret our results to indicate that *Mu* elements transpose at an extremely high rate and by a replicative mechanism. Copies of *Mu* segregate with expected ratios to progeny, whereas newly transposed copies appear at a rate of about 10–15 new *Mu* copies per genome per generation; *Mu* elements do not leave their previous site as an obligatory part of the transposition process. This suggests that transposition occurs by a replicative mechanism (SHAPIRO, ADHYA and BUKHARI 1977) as opposed to a lambda-like excision-transposition model (CAMPBELL 1983). According to a replicative transposition model, new *Mu* copies would arise via replication of old copies that do not leave their chromosomal position (SHAPIRO 1979). It is not possible to show whether an excision-transposition mechanism occurs at all. However, excision of *Mu1* from the first intron of *Adh1* in the mutant *Adh1-1S3034* would create revertants or mutant *Adh1* derivatives (*i.e.*, *Mu1* leaves the *Adh1* locus, but produces a deletion with a resulting change of phenotype). Reversion of the *Mu*-bearing mutant allele, *Adh1-1S3034*, occurs at a frequency of approximately 10^{-5} revertants per pollen grain (FREELING, CHENG and ALLEMAN 1982). Derivative alleles of *S3034* also occur rarely, and none of them appear to have lost the *Mu1* element (STROMMER *et al.* 1982; TAYLOR and WALBOT 1985; M. ALLEMAN and M. FREELING, unpublished results).

The molecular behavior of the *Mu* transposons that we report here is in agreement with published descriptions of the mutagenesis and behavior of the Mutator lines. The frequency of *Mu* transposition events of 10–15 transpositions per gamete per generation is high enough to account for the observed mutation rate in Mutator lines. Using an average gene size of $3–5 \times 10^3$ bp and an estimate of 6×10^9 bp for the size of the maize genome, a specific gene would represent a target size of $5–8 \times 10^{-7}$ of the genome. Ten to fifteen transpositions of *Mu* elements per gamete per generation would suggest an insertion frequency of a *Mu* element into a specific gene of 5.0×10^{-6} to 1.25×10^{-5}. ROBERTSON has estimated the mutation frequency for specific genes in a Mutator background to be approximately 4.0×10^{-6} to 7.5×10^{-5} mutants per gamete (ROBERTSON 1985). This mutation frequency might result from the transposition of *Mu* elements into genes with scorable phenotypes. Our estimate of the putative rate of *Mu* transposition into genes and ROBERT-

SON's published mutation frequencies for genes in Mutator stocks agree closely.

We have shown that the number of *Mu* elements per genome does not change when Mutator lines are crossed to non-*Mu* lines, but does increase in the first generation of self-pollination. Copy number stabilizes during subsequent self-pollination, and the transposition frequency of *Mu* elements is greatly decreased. Assuming that mutagenesis is a reflection of transposition of *Mu* elements, there is agreement between these molecular data and the genetic results. Mutagenesis in Mutator lines is highest during outcrossing, but decreases during inbreeding of Mutator lines (ROBERTSON 1983).

Genetic analysis has determined that the timing of mutational activity in Mutator lines is very late in the development of the germ line or immediately after fertilization (ROBERTSON 1980, 1981b). Our results for *Mu* transposition suggest that most transpositions of *Mu* elements probably occur during the gametophyte stage and before fertilization. Our reasoning is as follows: copy number control of *Mu* transposition would act to maintain the number of *Mu* elements in continuously self-pollinated Mutator stocks, although the *Mu* copy number doubles in the first generation of self-pollination. Postmeiotic transposition of *Mu* elements would increase the copy number to the approximate value that existed before the meiotic reductive division. Progeny of an outcross to a non-*Mu* line would then have the same copy number as did their parent and the progeny generated from a self-pollination would contain twice as many *Mu* elements as did the parent. The reduction in copy number per cell that follows meiosis might activate transposition and result in the maintenance of copy number.

On the other hand, if control of copy number and transposition occurred after fertilization or early in the development of the sporophyte, we would expect the following: After meiosis and fertilization in a plant with 20 *Mu* elements (for example), an outcross would generate zygotes with 10 *Mu* elements. The zygotes, following a self-pollination, would have 20 copies of *Mu*. A copy number control mechanism that maintained 20 *Mu* copies would rectify the number to 20 in the progeny of outcrossed Mutator plants. There would be no increase in *Mu* copy number observed in the progeny of self-pollinated plants. Our results are clearly consistent with transposition and copy number control before fertilization.

The observed regulation of copy number that we report here may be due to interactions among factors involved in the transposition process, including putative transposase(s) and repressor(s) of transposition. These interactions would determine both the frequency and the timing of transposition. Copy number control has been observed for the Tn*5* transposon of *E. coli*; a decrease in the frequency of transposition in strains with many Tn*5* elements is partially due to the increased concentration of the Tn*5*-encoded transposition repressor (JOHNSON and REZNIKOFF 1984). Such a model has also been proposed for the *Ac* and the *En* elements in maize, based on the genetic evidence that specific deletions in these elements remove the autonomous transposition capability (FEDOROFF, WESSLER and SHURE 1983; PEREIRA *et al.* 1985). The dependence of a copy number control mechanism on the absolute *Mu* copy number might

result from a transposase and/or a transposition repressor specific to *Mu* being encoded by the Mutator line, but not necessarily by the *Mu* elements themselves.

We thank JOHN DOCTOR for helpful criticism of this manuscript and JUDITH N. STROMMER for instruction in blot hybridizations. We also thank ELLEN DANIELL for the *Mu* subclone, pMuED2. Supported by a National Institutes of Health grant (GM27134) to M.F. M.A. was a recipient of a Public Health Services Genetics training grant and was an ARCO Plant Cell Research Institute fellow.

LITERATURE CITED

BARKER, R. F., D. V. THOMPSON, D. R. TALBOT, J. A. SWANSON and J. L. BENNETZEN, 1984 Nucleotide sequence of the maize transposable element *Mu1*. Nucleic Acids Res. **12:** 5955–5967.

BENNETZEN, J., 1984 Transposable element *Mu1* is found in multiple copies only in Robertson's Mutator maize lines. J. Mol. Appl. Genet. **2:** 519–524.

BENNETZEN, J., J. SWANSON, W. C. TAYLOR and M. FREELING, 1984 DNA insertion in the first intron of maize *Adh1* affects message levels: cloning of progenitor and mutant *Adh1* alleles. Proc. Natl. Acad. Sci. USA. **81:** 4125–4128.

CAMPBELL, A., 1983 Bacteriophage lambda. pp. 65–103. In: *Mobile Genetic Elements*, Edited by J. SHAPRIO. Academic Press, New York.

DENNIS, E. S., W. L. GERLACH, A. J. PRYOR, J. L. BENNETZEN, A. INGLIS, D. LLEWELLYN, M. M. SACHS, R. J. FERL and W. J. PEACOCK, 1984 The *Adh1* gene of maize. Nucleic Acids Res. **12:** 3983–4000.

FEDOROFF, N. V., 1983 Controlling elements in maize, pp. 1–63. In: *Mobile Genetic Elements*, Edited by J. SHAPIRO. Academic Press, New York.

FEDOROFF, N. V., S. WESSLER and M. SHURE, 1983 Isolation of the transposable maize controlling elements *Ac* and *Ds*. Cell **35:** 235–242.

FREELING, M., 1984 Plant transposable elements and insertion sequences. Annu. Rev. Plant Physiol. **35:** 277–298.

FREELING, M., D. S.-K. CHENG and M. ALLEMAN, 1982 Mutant alleles that are altered in quantitative, organ-specific behavior. Dev. Genet. **3:** 179–196.

GREEN, M. M., 1977 The case for DNA insertion mutants in Drosophila. pp. 437–445. In: *DNA Insertion Elements, Plasmids, and Episomes*, Edited by A. I. BUKHARI, J. A. SHAPIRO and S. L. ADHYA. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

JOHNS, M. A., M. ALLEMAN and M. FREELING, 1983 Differential regulation of the *Adh1* gene in maize: facts and theories. pp. 61–79. In: *Genetic Engineering of Plants*, Edited by T. KOSUGE, C. MEREDITH and A. HOLLAENDER. Plenum Press, New York.

JOHNS, M. A., J. N., STROMMER and M. FREELING, 1983 Exceptionally high levels of restriction site polymorphism in DNA near the maize *Adh1* gene. Genetics **105:** 733–743.

JOHNSON, R. C. and W. S. REZNIKOFF, 1984 Copy number control of *Tn5* transposition. Genetics **107:** 9–18.

MCCLINTOCK, B., 1950 The origin and behavior of mutable loci in maize. Proc. Natl. Acad. Sci. USA **36:** 344–355.

MURRAY, M. G. and W. F. THOMPSON, 1980 Rapid isolation of high molecular weight plant DNA. Nulcleic Acids Res. **8:** 4321–4325.

O'REILLY, C., N. S. SHEPHERD, A. PEREIRA, Z. SCHWARZ-SOMMER, I. BERTRAM, D. S. ROBERTSON, P. A. PETERSON and H. SAEDLER, 1985 Molecular cloning of the *a1* locus of *Zea mays* using the transposable elements *En* and *Mu1*. EMBO J. **4:** 877–882.

PEREIRA, A., Z. SCHWARZ-SOMMER, A. GIERL, I. BERTRAM, P. A. PETERSON and H. SAEDLER, 1985 Genetic and molecular analysis of the enhancer (*En*) transposable element system of *Zea mays*. EMBO J. **4:** 17–23.

PETERSON, P. A., 1960 The pale green mutable locus in maize. Genetics **45:** 115–133.

RHOADES, M. M., 1938 Effect of the *Dt* gene on the mutability of the *a1* allele in maize. Genetics **23:** 377–397.

RIGBY, P. W., M. DIECKMANN, C. RHODES and P. BERG, 1977 Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation. J. Mol. Biol. **113:** 237–251.

ROBERTSON, D. S., 1978 Characterization of a mutator system in maize. Mutat. Res. **51:** 21–28.

ROBERTSON, D. S., 1980 The timing of *Mu* activity in maize. Genetics **94:** 969–978.

ROBERTSON, D. S., 1981a Mutator activity in maize: timing of its activation in ontogeny. Science **213:** 1515–1517.

ROBERTSON, D. S., 1981b Tests of two models for the transmission of the (*Mu*) Mutator in maize. Mol. Gen. Genet. **183:** 51–53.

ROBERTSON, D. S., 1983 A possible dose dependent inactivation of Mutator (*Mu*) in maize. Mol. Gen. Genet. **191:** 86–90.

ROBERTSON, D. S., 1985 Differential activity of the maize Mutator (*Mu*) at different loci and in different cel lineages. Mol. Gen. Genet. **200:** 9–13.

ROBERTSON, D. S. and P. N. MASCIA, 1981 Tests of four controlling element systems of maize for Mutator activity and their interaction with (*Mu*) Mutator. Mutat. Res. **84:** 283–289.

SHAPIRO, J. A., 1979 Molecular model for the transposition and replication of bacteriophage *Mu* and other transposable elements. Proc. Natl. Acad. Sci. USA **76:** 1933–1937.

SHAPIRO, J. A., S. L. ADHYA and A. I. BUKHARI, 1977 Introduction: new pathways in the evolution of chromosome structure. pp. 3–12. In: *DNA Insertion Elements, Plasmids, and Episomes*, Edited by A. H. BUKHARI, J. A. SHAPIRO and S. L. ADHYA. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

STROMMER, J. N., S. HAKE, J. BENNETZEN, W. C. TAYLOR and M. FREELING, 1982 Regulatory mutants of the maize *Adh1* gene caused by DNA insertions. Nature **300:** 542–544.

TAYLOR, L. P. and V. WALBOT, 1985 A deletion adjacent to the maize transposable element *Mu1* accompanies loss of *Adh1* expression. EMBO J. **4:** 869–876.

Communicating editor: W. F. SHERIDAN