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

MARY ALLEMAN\(^1\) AND MICHAEL FREELING\(^2\)

*Department of Genetics, University of California, Berkeley, California 94720*

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**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 IS2P, 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

\(^1\) Present address: Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706.

\(^2\) To whom reprint requests should be addressed.
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, shl-mu9626, (D. S. Robertson, B. Burr and M. Freeling, personal communication) and a mutant in anthocyanin biosynthesis, al-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, al-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 X 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
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 N2 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 Tth111I, 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. Danielll. The plasmid pMuED2 consists of a Sau3AI/HindIII 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 Adhl-IS3034 genomic clone containing the Mu1 transposable element of maize. Mu1 was isolated from the unstable Mutator-induced mutant of the Adhl gene (Adhl-IS3034) (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 Adhl sequence.

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

Mu1-containing plasmid DNA or Adhl DNA was nick-translated to a specific activity of at least 10⁸ cpm/μg DNA with α-¹⁴C 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 0.2X 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, Tth111-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 (Hinfl and Tth111-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 Tth111-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 × 10⁹ 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 Tth111-I. DNA gel blots of 0.8% agarose gels were probed with the nick-translated MuI-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 MuI-containing plasmid DNA diluted to produce 1, 3, 5, 20, 30 and 50 genomic equivalents of MuI. 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 MuI-hybridizable sequences per genome for each plant. These copy number controls consisted of a MuI subclone, digested under two restriction enzyme regimes. Plasmid was digested with Tth111-I to produce a MuI fragment of 1.0 kbp, or with PstI and HindIII to yield a MuI-containing fragment of 1.7 kbp (see Figure 2). The Tth111-I and the PstI/HindIII 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 EcoRI 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 (Benetzen 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 SacI, 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
striping (FREELING 1984) and the induction of new mutants, occurred in this lineage. Copy number maintenance and mutagenesis in this stock indicate that \textit{Mu2} is not necessary for Mutator activity or for transposition of \textit{Mul}. Lane 12 of Figure 3 shows a DNA gel blot profile of DNA from an active Mutator plant containing no \textit{Mu2} elements.

\textbf{Mu sequences in stocks that have lost Mutator activity:} ROBERTSON (1978) observed that, following crosses of Mutator by non-Mutator plants, approximately 10\% 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 $\times$ 1070-8 and eight generations of outcrosses to non-\textit{Mu} stocks; others are individuals 2-4 and 2-16 (Figure 1) and their progeny. These stocks lose \textit{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 \textit{Mu} elements. Individual 2-16 had 12 copies of \textit{Mu}, approximately one-half of the number detected in its parent. This rate of loss is consistent with segregation of \textit{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 \textit{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 \textit{Mu} transposons and the Robertson’s Mutator phenomenon.

The data presented above show that, in the IS2P;Mutator lineage, approximately 10–15 new \textit{Mu} insertions are detected per generation during sequential outcrossing of Mutator lines. This is equivalent to approximately one trans-
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-IS3034 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-IS3034, 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 × 10³ bp and an estimate of 6 × 10⁹ bp for the size of the maize genome, a specific gene would represent a target size of 5–8 × 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 Tn5 transposon of E. coli; a decrease in the frequency of transposition in strains with many Tn5 elements is partially due to the increased concentration of the Tn5-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 \textit{Mu} being encoded by the Mutator line, but not necessarily by the \textit{Mu} elements themselves.

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