The Heritable Activation of *cryptic Suppressor-mutator* Elements by an Active Element

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ABSTRACT

A weakly active maize *Suppressor-mutator* (*Spm-w*) element is able to heritably activate *cryptic Spm* elements in the maize genome. The spontaneous activation frequency, which is $1-5 \times 10^{-5}$ in the present genetic background, increases by about 100-fold in the presence of an *Spm-w* and remains an order of magnitude above the background level a generation after removal of the activating *Spm-w*. Sectorial somatic reactivation of *cryptic* elements can be detected phenotypically in kernels. Selection of such kernels constitutes an efficient selection for plants with reactivated *Spm* elements. Analysis of the reactivation process reveals that it is gradual and proceeds through genetically metastable intermediates that exhibit different patterns of element expression during plant development. Newly reactivated elements tend to return to an inactive form. However, the probability that an element will remain in a heritably active state increases when the element is maintained in the presence of an active *Spm* element for several generations.

IT has been evident for some time that the maize genome contains genetically cryptic transposable elements. McCLINTOCK's studies (1945–1948) on transposable elements commenced from the observation that new unstable mutations occur frequently in plants with broken chromosomes. Both McCLINTOCK and DOERSCHUG later showed that the activation of cryptic elements could be enhanced systematically by the introduction of broken chromosomes into the genome (McCLINTOCK 1950; DOERSCHUG 1973). It has subsequently become increasingly apparent that agents and manipulations that cause chromosome damage, either directly or indirectly, stimulate the activation of cryptic elements. These include X-, UV-, and γ-irradiation, tissue culture, virus infection and mutagenic chemicals, such as EMS (NEUFFER 1966; SPRAGUE and McKINNEY 1966, 1971; BIANCHI, SALAMINI and RESTAINO 1969; FRIEDMANN and PETERSON 1982; PETERSON and FRIEDMANN 1983; DELLAPORTA *et al.* 1984; PESCHKA, PHILLIPS and GENGENBACH 1985; WALBOT 1988; BURR and BURR 1988). The results of molecular studies on maize transposable elements have begun to provide evidence that element sequence methylation is central to the molecular mechanism by which elements are maintained in a genetically silent state (FEDOROFF, WESSLER and SHURE 1983; BENNETZEN 1985; SCHWARTZ and DENNIS 1986; CHANDLER and WALBOT 1986; CHOMET, WESSLER and DELLAPORTA 1987; FEDOROFF *et al.* 1988; BANKS, MASSON and FEDOROFF 1988).

Our recent studies have focused on the *Suppressor-mutator* (*Spm*) element of maize (MASSON *et al.* 1987; FEDOROFF *et al.* 1988; FEDOROFF and BANKS 1988; BANKS, MASSON and FEDOROFF 1988). McCLINTOCK's genetic studies on the *Spm* element family, as well as those of PETERSON on the cognate *Enhancer* (*En*) element, revealed the existence of different types of interactions between the fully functional element of the *Spm* family and genes with mutations caused by the insertion of transposition-defective *Spm* (*dSpm*) elements (reviewed in McCLINTOCK 1965; FEDOROFF 1983). Most mutant alleles with *dSpm* insertions belong to one of two groups of alleles, which we have designated *Spm-suppressible* and *Spm-dependent* (MASSON *et al.* 1987). These designations reflect the ability of the inserted *dSpm* element to mediate either the inhibition or activation, respectively, of expression of the mutant gene in the presence of a *trans*-acting, fully functional *Spm* element. The results of studies on *Spm-dependent* alleles of the *a* locus suggested that the element encodes a positive autoregulatory gene product which maintains the element in a genetically active form (MASSON *et al.* 1987).

In order to test this hypothesis, we undertook studies on the genetic mechanism responsible for both differential patterns of element expression in development and the genetically stable, albeit reversible, inactivation of the element (McCLINTOCK 1957, 1958, 1965, 1971; PETERSON 1966; FOWLER and PETERSON 1978). We reported that the genetic mechanism underlying the element's developmental control comprises two components, one of which determines the element's *phase* of activity (active or inactive), and the other of which determines the heritability of the element's activity phase, as well as when, where and at what frequency the phase will be reversed during

development (FEDOROFF and BANKS 1988). In these studies, we characterized three element forms: (1) a stably active form, (2) a stably inactive form, termed *cryptic*, and (3) an unstable form termed *programmable*, in which the element displays one of a variety of heritable developmental expression programs (FEDOROFF and BANKS 1988; BANKS, MASSON and FEDOROFF 1988). We reported that *cryptic*, inactive *programmable* and active elements differ in the methylation level of cytosine residues within a short sequence in the immediate vicinity of the element's transcription start site, but not elsewhere in its sequence (BANKS, MASSON and FEDOROFF 1988).

The results of these studies also provided direct evidence for the existence of an element-encoded positive regulator which is able to *trans*-activate the transcription of an inactive *programmable* element, but not that of a *cryptic* element (FEDOROFF and BANKS 1988; BANKS, MASSON and FEDOROFF 1988). We further observed that exposure of an inactive *programmable Spm* to an active element had the effect of markedly reducing methylation of the element's 5′ end and increasing the probability that the *trans*-activated element would remain in a heritably active form after segregating from the active element (BANKS, MASSON and FEDOROFF 1988). We found that although fully methylated *cryptic Spm* elements were not transcriptionally *trans*-activated by an active element, they nonetheless exhibited a partial and variable reduction in the extent of methylation in the presence of the active element. This observation suggested that an element-encoded gene product can directly interfere with the maintenance of the *cryptic* element's methylation level. If there is a causal connection between the extent of methylation of the element's 5′ end and its genetic activity, a *trans*-activating element should promote the conversion of a *cryptic* element to an active element, albeit at a much lower frequency than it is able to activate an *Spm* element in the labile inactive *programmable* form (BANKS, MASSON and FEDOROFF 1988).

To test the foregoing hypothesis, I investigated the ability of a weakly active *Spm* (*Spm-w*) to affect the probability of activation of *cryptic* elements in the maize genome. I present evidence that the presence of an *Spm-w* element enhances the probability of activation of a *cryptic* element by 2–3 orders of magnitude and that this effect of the *Spm-w* element persists after the activating element is removed by genetic segregation. I further show that the activation of a *cryptic* element is gradual, occurring over several generations in the presence of a *trans*-activating *Spm-w*, and proceeds through the labile, readily *trans*-activatable, programmable forms identified in earlier studies (FEDOROFF and BANKS 1988; BANKS, MASSON and FEDOROFF 1988). I have identified newly activated elements with different developmental expression programs and show that a given program is subject to further modification. My results suggest that genetic activation of a *cryptic Spm* is a gradual process that proceeds through a series of genetically metastable states and provide evidence that an element-encoded gene product promotes the transition from the cryptic to the active form.

MATERIALS AND METHODS

Genetic strains: All of the *a-m5w* lines used in the present study were derived from a single culture obtained from B. MCCLINTOCK (1962). The *a-m5w* allele was derived by MCCLINTOCK from the original *a-m5* allele (MCCLINTOCK, 1962) and has a weakly active *Spm* element inserted within the coding sequence at the *a* locus (N. FEDOROFF and J. A. BANKS, unpublished data). The frequency at which the allele reverts to *A* or mutates to either a pale or colorless stable allele is <0.1% (FEDOROFF 1986). Most of the plants used in the present study had the genetic constitution *a-m5w Sh2/a sh2* and were homozygous for the *wx-m8* allele of the *waxy* (*wx*) locus. The recombination frequency between the *a* and *shrunken 2* (*sh2*) loci is <0.2%. The *Sh2* allele therefore served as a segregation marker for the *a-m5w* allele. The two tester alleles with transposition-defective *Spm* (*dSpm*) insertion mutations used were the *wx-m8* allele of the *wx* locus and the *a-m1-5719A1* allele of the *a* locus (MCCLINTOCK 1951, 1961). Both have *dSpm* insertions that are excised in the presence of a *trans*-acting *Spm-s* or *Spm-w* element (Figure 1h). The *wx-m8* allele has a null phenotype in the absence of a *trans*-acting *Spm* element, while the *a-m1-5719A1* allele is an *Spm-suppressible* allele (MASSON *et al.* 1987) and exhibits a level of pigmentation intermediate between the wild-type, deeply pigmented *A* allele and the recessive null *a* allele (Figure 1e). The backcross tester parents used in the present study were all homozygous for the *wx-m8* allele and for one of the following chromosome *3* constitutions: *a sh2, a-m1-5719A1 Sh2*, or *a-m1-5719A1 sh2*. None of the alleles used in these studies is in standard inbred backgrounds. All of the maize genomic DNA samples tested contain many copies of sequences with homology to the *Spm* element (J. BANKS and N. FEDOROFF, unpublished data), but their content of full length, genetically silent *Spm* elements is not known.

Scoring of ears: Ears obtained as a result of the crosses described in the figure legends were analyzed for evidence of *Spm-s* activity. The *Sh2* class of kernels, essentially all of which received the closely linked *a-m5w* allele, were examined for the presence of either sectors or full kernels with the high frequency of excision (*Hfe*) phenotype characteristic of the *a-m5w* allele in the presence of a standard *Spm* (*Spm-s*; FEDOROFF and BANKS 1988). Kernels without and with increasingly large *Hfe* sectors in the aleurone layer are shown in Figure 1, a–c. Kernels exhibiting *Hfe* sectors covering more than approximately ⅛ of the kernel surface were assigned to the "*Hfe* sector" category (Figure 1c), while those showing the *Hfe* phenotype throughout the aleurone were assigned to the "*Hfe*" category (Figure 1d). For the purposes of determining the percentage of kernels with *Spm-s* activity, the *Hfe* and *Hfe*-sector categories were summed. Kernels that did not receive the *a-m5w* allele after meiotic segregation were assessed for the presence of *Spm* activity after crosses to either an *a-m1-5719A1, wx-m8* tester or an *a, wx-m8* tester. Kernels having the *a-m1-5719A1* allele were examined for colorless areas containing deeply pigmented

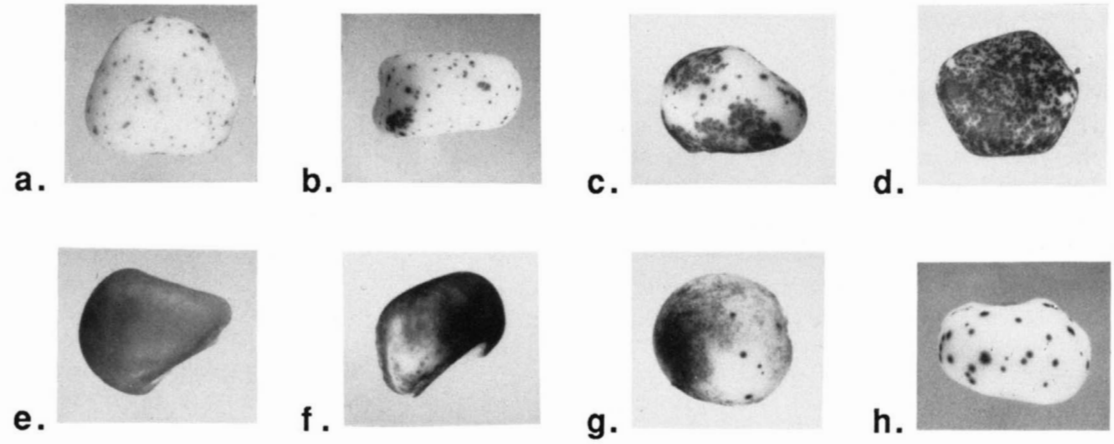


FIGURE 1.—Phenotypes of the aleurone layer in kernels carrying the *a-m5w* allele or the *a-m1-5719A1* allele. a, *a-m5w*; b and c, *a-m5w* with small and large *Hfe* sectors indicative of *Spm-s* activity; d, *a-m5w* exhibiting the *Hfe* phenotype throughout the aleurone layer, indicating early activation of a *cryptic Spm-s* element; e, *a-m1-5719A1*, no *Spm*; f and g, *a-m1-5719A1* with *Spm-s* activity in sectors of the aleurone; h, *a-m1-5719A1* with a fully active *Spm-s*.

revertant *A* sectors (Figure 1, f and g). Kernels having only the *wx-m8* allele were either examined visually for the presence of translucent *Wx* sectors or cut and stained with an iodine-potassium iodide solution to detect deeply staining *Wx* sectors. For the purposes of the present analysis, all kernels which showed more than one revertant sector were assigned to the category exhibiting *Spm* activity. The results are expressed as the percentage of kernels in each of the classes defined by the segregating *a-m5w* allele that exhibit evidence of *Spm* activity. A single *Spm* element located on a chromosome other than chromosome *3* will give *Spm* activity in 50% of each kernel class defined by the segregation of the *a-m5w* allele.

RESULTS

Spontaneous reactivation of cryptic *Spm* elements is an infrequent event: The spontaneous activation of genetically cryptic elements can be monitored conveniently using a mutant allele with a transposition-defective *Spm* element (*dSpm*) insertion. Because an *Spm* element can *trans*-activate excision of a *dSpm* element, excision of the inserted *dSpm* element permits the detection of a newly activated element anywhere in the genome. In the present study, we have used the *a-m1-5719A1* allele of the *a* locus (Figure 1, e and h) and the *wx-m8* allele of the *waxy* (*wx*) locus, both of which are expressed in kernel endosperm tissue and therefore permit the evaluation of large numbers of progeny.

Among the 59 ears (19,816 kernels) carrying the *a-m1-5719A1* tester allele that were examined, one kernel exhibited a single small sector of the colorless, variegated phenotype characteristic of the tester allele in the presence of an active *Spm* (Figure 1f). All of the remaining kernels had the uniformly palely pigmented phenotype of the allele in the absence of a *trans*-activating *Spm* element (Figure 1e). In a previous study, two kernels with weakly active *Spm* elements (*Spm-w*) were recovered from among the more than 100,000 kernels examined (FEDOROFF 1986). Although the number of activation events is too low to provide an accurate measure of the spontaneous reactivation frequency, it appears to be in the range of $1-5 \times 10^{-5}$ in the present genetic background. BURR and BURR (1988) have reported a similar value in a somewhat different genetic background.

The activation of cryptic elements can be monitored using an *Spm-w* element: *Spm-w* elements were first identified by MCCLINTOCK (1957) as derivatives of a *standard Spm* (*Spm-s*) that transposed and promoted transposition of *dSpm* elements less frequently and much later in development than the parent *Spm-s*. The results of molecular analyses of two *Spm-w* elements showed them to be internally deleted (MASSON *et al.* 1987; GIERL *et al.* 1988). It has also been reported that the element's major 2.5-kb transcript is much less abundant in tissue of plants with an *Spm-w* element than of plants with an *Spm-s* element (MASSON *et al.* 1987; BANKS, MASSON and FEDOROFF 1988). The genetic property of an *Spm-w* element that is important in the present context is that it exhibits an extremely low frequency of germinal transposition. Among the several alleles with *Spm-w* insertions used in the present study, the *a-m5w* allele was used most extensively. The phenotype of this allele is shown in Figure 1a. The *Spm-w* insertion in this allele is quite stable. Germinal excision of the element to give either revertant *A* alleles or colorless, stable *a* alleles occurs at a frequency of less than 0.1% (FEDOROFF 1986).

Many kernels on ears of plants that carry the *a-m5w* allele exhibit densely variegated sectors (Figure 1, b and c). Moreover, a few kernels on each ear exhibit a densely variegated phenotype throughout the kernel aleurone layer (Figure 1d). Since the variegation is due to the restoration of normal *a*-gene expression after excision of the element in the progenitor cell

that gave rise to the pigmented cell clone, the increased density of variegation signals an increase in the number of cells in which excision has occurred. Moreover, kernels and kernel sectors showing a high frequency of excision of the *Spm* element residing at the *a* locus also show an increase in the excision frequency of the *dSpm* element insorted at the *wx* locus in the *wx-m8* allele present in all of the plants.

The high frequency of excision (*Hfe*) phenotype observed occasionally on ears carrying the *a-m5w* allele is regularly observed when an *Spm-s* element is present together with the *a-m5w* allele. This is because the *Spm-s* element *trans*-activates excision of the *Spm-w* element, just as it can *trans*-activate excision of a *dSpm* element (McCLINTOCK 1962; FEDOROFF and BANKS, 1988). Thus the regular, albeit infrequent, appearance of *Hfe* kernels on ears of plants carrying only the *a-m5w* allele may signal the reactivation of *cryptic Spm* elements in the genome. To investigate this possibility, plants were grown from kernels assigned to three different phenotypic categories based on the *Hfe* phenotype. These were (1) kernels that exhibited either no *Hfe* sectors or sectors occupying less than roughly 1/8 of the kernel surface, (2) kernels with large *Hfe* sectors, occupying more than 1/8, but not all of the kernel surface, and (3) fully *Hfe* kernels.

Kernels were selected from plants that carried the dominant *Sh2* allele of the *sh2* locus linked to the *a-m5w* allele of the *a* locus. The selected kernels were also heterozygous for the recessive *a* and *sh2* alleles (*a-m5w Sh2/a sh2*) and homozygous for the *wx-m8* allele. Plants grown from such kernels were crossed to and by testers that were either homozygous for the *a, sh2* and *wx-m8* alleles, or for the *a-m1-5719A1, sh2,* and *wx-m8* alleles. The resulting ears were scored for the percentage of *Hfe* kernels and kernels with large *Hfe* sectors in the *a-m5w Sh2* class (+*Spm-w*), as well as kernels showing variegation for the *dSpm* allele present in the *sh2* class (−*Spm-w*).

Ears produced on plants grown from kernels showing no *Hfe* sectors or only small *Hfe* sectors are similar to the parent ears from which they were selected. That is, most kernels exhibit either no *Hfe* sectors or a few small *Hfe* sectors. A small fraction of kernels exhibits large *Hfe* sectors and a few kernels show the *Hfe* phenotype throughout the aleurone layer. The fraction of such kernels varies from plant to plant within a family of plants grown from kernels selected from a single ear, as well as among sibling families. The average fraction of kernels with a full *Hfe* phenotype or large *Hfe* sectors is given in Table 1A for 11 different families of plants. As is evident from the average values, there is a heritable component in the variation. The tendency to produce ears with a large fraction of *Hfe* kernels is observed in two families (2054, 2055), while one family showed almost no such kernels (2037). Most families had an average of 1–4% *Hfe* or *Hfe*-sectored kernels.

Data for the 96 ears (19,373 +*Spm-w* kernels) analyzed in the experiment reported in Table 1A were grouped by the percentage of kernels on each ear exhibiting *Spm-s* activity, as judged by the appearance either of large *Hfe* sectors or the *Hfe* phenotype throughout the aleurone layer. The resulting histogram is shown in the left panel of Figure 2a. Most of the ears (92.9%) exhibit a low frequency (<10%) of *Hfe* and *Hfe*-sectored kernels. A small fraction of the ears (7.1%) exhibited higher frequencies of such kernels, although the frequency did not exceed 35% in any of the ears examined.

Within the same families of plants, the frequency of kernels that did not receive the *Sh2* allele after meiotic segregation, but show evidence of *Spm* activity is low. It is nonetheless sporadically well above the range expected for recombination between the *a* and *sh2* loci (<0.2%). Family 2055 (Table 1A) was exceptional in this regard, showing variegated sectors or a fully variegated phenotype in 5.9% of the kernels. The pooled data from the 86 ears (15,981 kernels) in which the class of kernels not receiving the *Spm-w* element at the *a* locus was examined for *Spm* activity are summarized in the right histogram of Figure 2a. When the right and left panels of Figure 2a are compared, it is apparent that fewer ears (2.5%) gave evidence of *Spm* activity in the class of kernels that did not receive the *a-m5w* allele (right panel, −*Spm-w*), than in the class of kernels that received the allele (7.1%; left panel, +*Spm-w*). In no case did the frequency of variegated kernels exceed 16% in the class of kernels that did not receive the *Spm-w* element.

The results of comparable analyses carried out on plants grown from kernels exhibiting large *Hfe* sectors and the *Hfe* phenotype throughout the aleurone layer are presented in the form of histograms in parts b and c of Figure 2, respectively. Kernels were selected from ears grown from plants which had been similarly selected in the previous year and shown slightly higher frequencies of kernels with the *Hfe* phenotype than had the parental ears. Progeny ears produced by plants grown from such kernels showed a markedly higher proportion of *Hfe* kernels than the parental ears, which ranged from 0.3 to 3.6% *Hfe* kernels. Among the 30 progeny ears produced on plants grown from kernels exhibiting large *Hfe* sectors, 40% showed the *Hfe* phenotype in more than 10% of the kernels receiving the *Spm-w* element of the *a-m5w* allele (Figure 2b, left panel). Of these ears, half exhibited the *Hfe* phenotype in 40–60% of kernels, the frequency range expected if there is an independently segregating *Spm-s* element present. A smaller fraction (11.5%) of ears showed evidence of *Spm* activity in more than 10% of the class of kernels that did not

TABLE 1

Frequency of *Spm-s* activation in the presence and absence of a *trans*-acting *Spm-w* element

	A. *Spm-w* present, no *Hfe* phenotype					B. *Spm-w* present, *Hfe* kernels selected					C. No *Spm-w*		
		+*Spm*[b]		−*Spm*[b]			+*Spm*		−*Spm*				
Family[a]	Ear No.	Kernel No.	*Spm-s* activity[c]	Kernel No.	*Spm-s* activity[c]	Ear No.	Kernel No.	*Spm-s* activity[c]	Kernel No.	*Spm-s* activity[c]	Ear No.	Kernel No.	*Spm-s* activity[c]
2037	5	1129	0.32	1160	0.32	11	1802	4.0	543	0.14	83	12,422	0.016
2038	7	1688	3.90	1141	0.14	1	155	14.2			17	6,489	0.000
2040	12	1844	4.20	1849	0.00	2	413	2.2			17	6,492	0.000
2041	5	857	1.50	891	0.00	5	1001	1.4			12	3,220	0.031
2043	9	1800	1.20	1678	0.00	1	115	2.6			27	10,188	0.010
2045	8	1810	1.40	1730	0.08	3	573	9.0	290	0.00	14	6,583	0.390
2046	8	1628	3.10	1600	0.00	1	315	3.5			10	3,864	0.025
2047	12	2992	2.50	2943	0.10	1	172	37.0	171	0.60	18	8,482	0.012
2054	10	1554	7.20	1535	0.00	0					13	3,737	0.026
2055	5	973	16.50	954	5.90	5	995	16.2			16	6,694	0.045
2056	15	3098	3.90			0					17	7,051	0.000
Average:			4.20		0.65			10.0		0.25			0.050

[a] Each family comprised plants grown from kernels selected from a single ear. All of the families consisted of plants grown from kernels on ears of plants that had the genetic constitution *a-m5w Sh2/a sh2, wx-m8/wx-m8* and had been backcrossed by plants that were homozygous for the *a, sh2* or *Sh2* and *wx-m8* allele. The first part of each family, reported in part A of the table, comprised kernels that were variegated for *a* and *wx* expression, but showed the late and infrequent pattern of somatic reversion characteristic of the *a-m5w* allele (Figure 1a). The second part of each family, reported in part B, comprised the few kernels on each of the parental ears that showed the *Hfe* phenotype throughout the kernel aleurone and also showed frequent reversion of the *wx-m8* allele (Figure 1d). The third part of each family, reported in part C, comprised kernels that were colorless and showed no reversion of the *wx-m8* allele to *Wx* (no *Spm*).

[b] The +*Spm* and −*Spm* columns report the *Spm-s* activity in the kernels of backcrossed progeny ears that did and did not receive the *a-m5w* allele. The backcross parent used in part C was homozygous for the *a-m1-5719A1, Sh2* and *wx-m8* alleles, while the backcross parent used in part A was homozygous for the *a-m1-5719A1, sh2* and *wx-m8* alleles. Two backcross parents were used in part B. These were homozygous for either the *a* or the *a-m1-5719A1* alleles and both were homozygous for the *sh2* and *wx-m8* alleles.

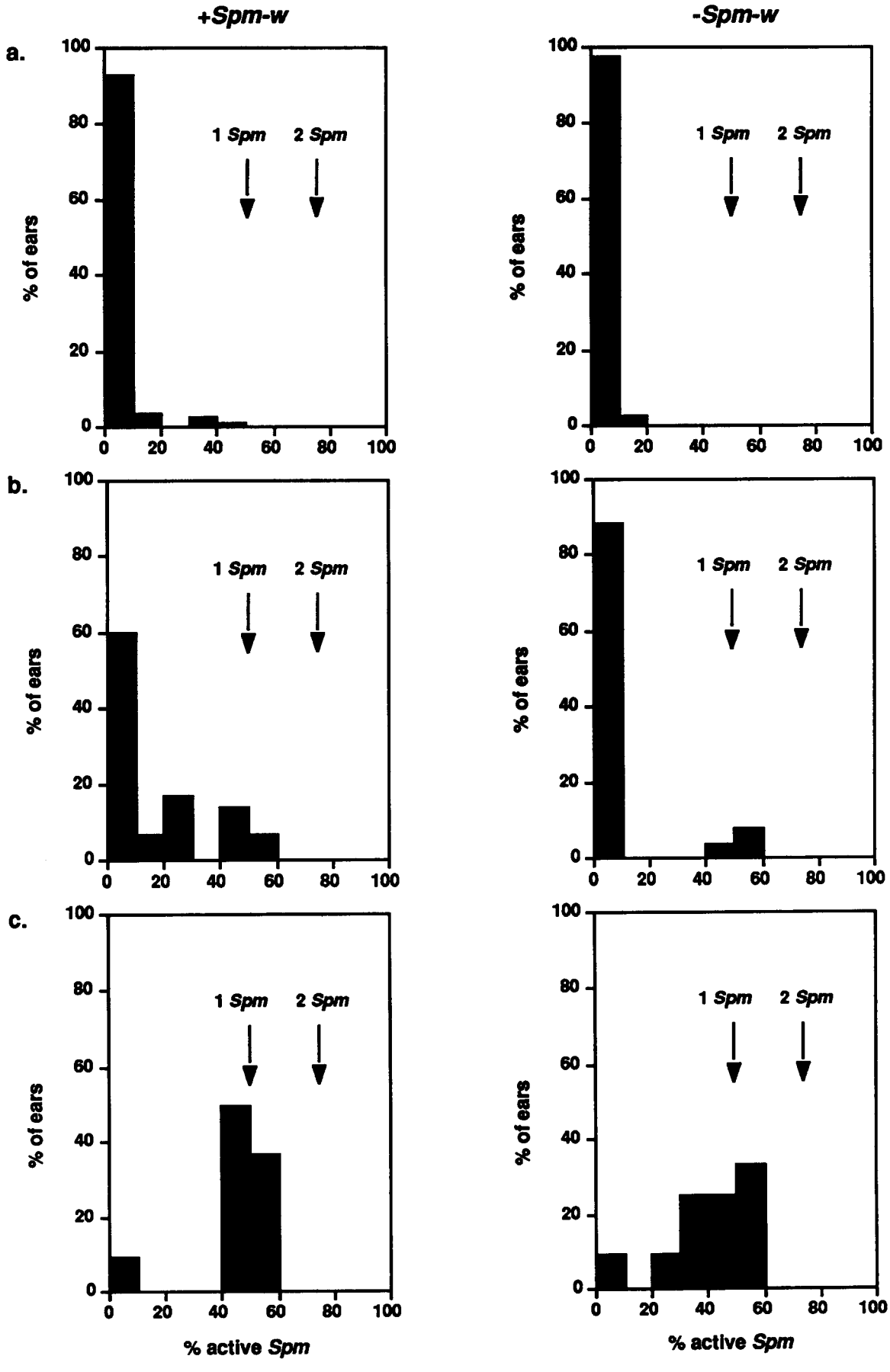
[c] The percentage of kernels with *Spm-s* activity comprises the sum of kernels showing *Hfe* sectors occupying more than about 1/8 of the kernels aleurone layer and kernels with an *Hfe* phenotype throughout the aleurone for those kernels that received the *a-m5w* allele. Among the kernels that did not receive the *a-m5w* allele (all of those in part C, as well as half of those in parts A and B), the percentage showing *Spm-s* activity included all kernels that showed reversion of either the *wx-m8* allele or the *a-m1-5719A1* allele in either a kernel sector or throughout the kernel endosperm or aleurone.

receive the *a-m5w* allele (Figure 2b, right panel). But the few ears that showed significant levels of *Spm* activity all exhibited *Spm* activity in 40–60% of the kernels, suggesting the presence of an *Spm-s* element segregating independently of the *Spm-w* at the *a* locus.

Among the 22 progeny ears produced on plants grown from kernels exhibiting the *Hfe* phenotype throughout the aleurone layer, 20 (91%) exhibited the *Hfe* phenotype in more than 10% of progeny kernels (Figure 2c, left panel). Moreover, the fraction of *Hfe* kernels for all of these ears fell in the 40–60% range, again indicating the presence of an independently segregating *Spm-s* element. The fraction of ears showing *Spm* activity in more than 10% of the kernels was comparable in this group of plants for the classes of kernels that did and did not receive the *Spm-w* element of the *a-m5-w* allele (Figure 2c, left and right panels). Nonetheless, only 58% of the ears exhibited *Spm* activity in 50–60% of the *sh2* kernels, while 33.3% of the ears showed *Spm* activity in 20–40% of kernels. That is, within a given ear, *Spm-s* activity is detectable in a larger percentage of the kernels that received the segregating *a-m5w* allele than of those that did not receive it.

The results obtained from comparable analyses of plants grown from fully *Hfe* kernels, but without an intermediate generation of *Hfe* sector selection, are similar to those obtained with plants grown from *Hfe*-sectored kernels after an intermediate selection step (Figure 2b). Data for the plants grown from such *Hfe* kernels are given in Table 1B. Despite the considerable variation from family to family, it is evident that the family averages are higher for plants grown from *Hfe* kernels than for plants grown from kernels without *Hfe* sectors (Table 1, A and B). At the same time, none of the 31 plants obtained from *Hfe* kernels in this group of families exhibited the *Hfr* phenotype in more than 37% of the kernels receiving the *a-m5w* allele.

Taken together, these results indicate that the *Hfe* sectors appearing on kernels carrying the *a-m5w* allele are sectors within which a *cryptic Spm-s* element has been activated. They further suggest that the *Spm-w* element of the *a-m5w* allele promotes the activation of the *Spm-s* element. Thus, for example, sectors with an *Spm-s* phenotype appear on kernels containing an *Spm-w* element at a much higher frequency than they do on kernels containing a *dSpm* insertion allele that can detect the presence of an *Spm* element, but is itself not a transposition-competent element. This bears the


+Spm-w
-Spm-w
a.
b.
c.
1 Spm
2 Spm
% of ears
% active Spm
0
20
40
60
80
100

implication that a gene product encoded by the *Spm-w* element promotes the appearance of such sectors of apparent *Spm* activation. Selection of kernels which show the putative *Spm-s* activity in a large fraction of the aleurone layer or throughout the aleurone layer results in a rapid increase in the fraction of plants that exhibit the *Hfe* phenotype in about half of the kernels that receive the *Spm-w* element. This is the expected outcome if the selected plants contain an active *Spm-s* element that is located on a chromosome other than that containing the *a-m5w* allele. Since the parental plants showed no evidence of an independently segregating *Spm-s*, it follows that the origin of the element is likely to be in the conversion of a *cryptic Spm* element residing in the genome to an active element. This conjecture is supported by the reproducibility of the phenomenon in different genetic backgrounds and with different *Spm-w* elements (N. FEDOROFF, unpublished data). It is also supported by the observation that kernels with an *Hfe* phenotype are more likely to yield plants with an independently segregating *Spm* element than are kernels which exhibit only sectors of the *Hfe* phenotype in the aleurone layer. This is because *Hfe* sectors arise after meiosis, while an *Hfe* phenotype throughout the kernel aleurone layer implies a genetic change at or before meiosis and therefore one that is likely to be represented in both the zygotic and endosperm genomes.

However, in all cases, fewer kernels exhibit *Spm* activity in the kernel class that did not receive the *Spm-w* element of the segregating *a-m5w* allele than in the kernel class that received it (Table 1, Figure 2). This implies that the newly activated *Spm-s* element is in an intermediate form whose activity is dependent on the presence of a *trans*-activating element. This observation, in turn, suggests that the newly activated element resembles the readily *trans*-activatable inactive programmable form identified in previous studies on *Spm* inactivation (BANKS, MASSON and FEDOROFF 1988).

The *Spm-w* element can heritably enhance the spontaneous activation frequency of a cryptic element: To determine whether a *trans*-activating element has a heritable effect on a cryptic genomic element, the spontaneous activation frequency of cryptic elements was determined in a large population of plants grown from kernels selected from ears on plants containing the *a-m5w* allele, but which did not receive the homolog bearing the *Spm-w* element following meiotic segregation. On the assumption that the genome of these plants contains a single cryptic element that is segregating independently of the *a* locus, half of such kernels would be expected to receive the cryptic element. Ears of plants grown from the selected kernels were crossed to and by plants homozygous for the *a-m1-5719A1* and *wx-m8* tester alleles. The tester plants were taken from a group that had previously been determined to have a frequency of spontaneous element activation of 5×10^{-5} or less. The results of these tests are reported in Table 1C.

Among the 194 ears (75,221 kernels) examined, the average family frequency of kernels with *Spm* activity was $2\text{–}5 \times 10^{-4}$. In 10 of the 11 families, the averages ranged from 0 to 0.045%, and a single family had an average of 0.39%. The average for the first 10 families was 1.7×10^{-4}, while the average for all 11 families was 5×10^{-4}. Thus the frequency of spontaneous element activation in the generation following the segregation of the activating *Spm-w* element remains higher than the spontaneous activation frequency observed in the same genetic background. Nonetheless, this value is about an order of magnitude lower than that observed in the segregating generation (Table 1A). It appears, therefore, that exposure of a *cryptic Spm* element to an active one can predispose it to spontaneous activation. The effect of exposure to an active *Spm* element is lower in the generation after the activating element is eliminated from the genome of a plant containing a cryptic element than in the segregating generation, but still above the background activation frequency (Table 1, A and C). This implies that the influence of the *trans*-activating *Spm-w* element persists for at least a generation.

Newly activated elements are less efficiently transmitted through male germ cells than through female germ cells: Transmission of a newly activated element through the first ear produced on the main stalk and through pollen was compared for plants grown from single kernels showing the *Hfe* phenotype

FIGURE 2.—Activation of *cryptic Spm* elements in the presence of an *Spm-w* element. The histograms show the percentage of ears in each group of cultures which showed *Spm-s* activity in the indicated percentage of kernels (percent active *Spm*). The percentages are shown separately in the left and right panels for the classes of kernels that received the *a-m5w* allele (+*Spm-w*) and those that did not (−*Spm-w*) following meiotic segregation. The plants on which the ears were produced all had the genetic constitution *a-m5w Sh2/a sh2, wx-m8/wx-m8*. Data are shown for the first ear produced on each plant and backcrossed to a tester that was either homozygous for the *a-m1-5719A1, sh2* and *wx-m8* alleles or the *a, sh2* and *wx-m8* alleles. Criteria for identifying kernels with *Spm* activity are described in Materials and Methods. The arrows in each panel point to the percentages of kernels with *Spm* activity expected (50% and 75%) if either 1 or 2 independently segregating *Spm* elements are present in the ear. The histograms in part a represent all of the data for which family averages are given in Table 1A. b and c, Kernels containing the *a-m5w* allele and exhibiting large *Hfe* sectors in the aleurone layer (part b) or the *Hfe* phenotype throughout the aleurone (part c) were selected from ears produced on plants grown from kernels that had exhibited large *Hfe* sectors in the previous generation.

TABLE 2

Percentage of *Hfe* kernels among progeny of plants grown from *Hfe* kernels and used as female and male parents[a]

A. First generation of *Hfe* selection				B. Second generation of *Hfe* selection			
Family	Plant	♀ Gametes	♂ Gametes	Family	Plant	♀ Gametes	♂ Gametes
1466	1	2.9	0	1457	1	50.0	48.9
	2	0	0		2	51.6	55.3
	3	9.4	0.8		3	56.3	49.4
	4	2.3	0		4	49.1	42.4
	5	2.1	0.7		5	45.5	44.4
	6	5.8	0.8		6	52.1	48.2
	7	2.6	0		7	55.5	52.4
	8	3.2	0.8		8	51.0	29.0
	9	8.1	0.9		9	47.3	50.9
	10	14.2	0.6		10	50.5	52.1
	11	2.2	0		11	55.2	45.5
	12	0	0		12	47.0	31.0
	13	1.0	0		13	49.2	43.7
	14	2.2	0		14	49.6	54.5
	15	1.7	0		15	54.5	46.1
	16	2.4	0.5		16	48.2	42.3
	17	2.6	0		17	8.0	0.4
	18	9.5	0		18	47.0	7.7
1464	1	9.2	0		19	0.8	0.0
	2	8.3	0.4		20	43.8	45.0
	3	3.5	0				
	4	37.0	12.0				
1461	1	45.0	1.2				
	2	20.4	3.8				
	3	14.2	4.2				

[a] Data are shown only for the first ear produced on the main stalk of the plant carrying the *a-m5w* allele (♀ gamete column) or on a tester plant that received pollen from the same plant (♂ gamete column).

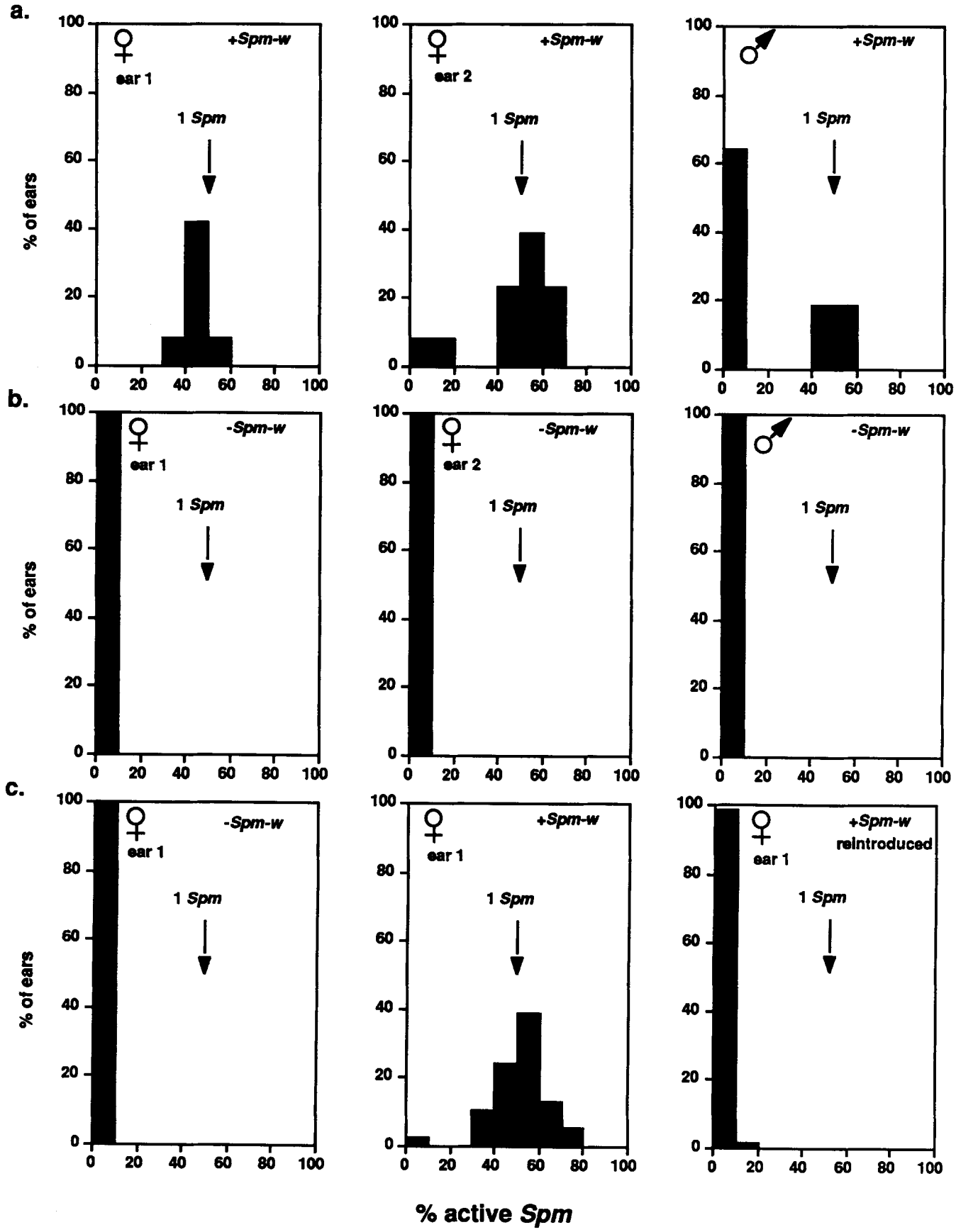
throughout the aleurone. The selections were carried out in four different families of plants, all of which were derived from two plants belonging to a single progenitor family carrying the *a-m5w* allele. Families 1461, 1464 and 1466 were grown from *Hfe* kernels taken from ears on plants grown from kernels that did not have prominent *Hfe* sectors (Table 2A), while family 1457 comprised plants grown from *Hfe* kernels selected from ears produced by plants that had been grown from selected *Hfe* kernels in the previous generation (Table 2B). Thus family 1457 represents the results obtained when selection for the *Hfe* trait was carried out in two successive generations.

A smaller percentage of kernels exhibited the *Hfe* phenotype when the *a-m5w* allele was transmitted through the pollen parent than when it was transmitted through the egg parent (Table 2). The asymmetric transmission of the newly activated element was much greater in the first generation of selection for the *Hfe* phenotype (Table 2A) than in the second generation of selection (Table 2B). Moreover, the fraction of kernels exhibiting the *Hfe* phenotype was higher after two generations of selection than after a single generation of selection. After two generations of selection, most ears (90%) exhibited the *Hfe* trait in about half of the kernels, the value expected if the ear contains a single active *Spm-s* element located on a chromosome other than the one bearing the *a-m5w* allele. These observations suggest that newly activated elements tend to return to an inactive state in the first generation, particularly upon transmission of the element through male gametes. The effectiveness of a second round of selection for kernels with the *Hfe* trait implies that the activation process is progressive and that complete activation of a *cryptic Spm* element requires more than a single generation.

A newly activated *Spm* element can exhibit the properties of a *Modifier* element: One plant (1466A-2) grown from a kernel with an *Hfe* phenotype subsequently proved to contain an independently segregating element with properties resembling those of the *Modifier* element described by McCLINTOCK (1957). In the first generation, the plant produced two ears on the main stalk. On the first and second ears, 45.6% and 16.1% of the kernels receiving the *a-m5w* allele had an *Hfe* phenotype, respectively. The same plant was used as a male parent in two crosses to ears on tester plants. There were almost no kernels (0.5%) with an *Hfe* phenotype on either ear. Moreover, in all four ears obtained, *Spm-s* activity was detected only in kernels that also received the *Spm-w* element of the *a-m5w* allele. Thus the element appeared to be active only in the presence of the *Spm-w* element, as is characteristic of previously isolated *Modifier* elements (McCLINTOCK 1957). However, the *Modifier*-like trait was detectable only when it was transmitted through the egg parent.

To assess the heritability of the *Modifier*-like trait, plants were grown from *Hfe* kernels selected from the

FIGURE 3.—The percentage of ears exhibiting *Spm-s* activity among the first and second generation progeny of plant 1466A-2. a, The percentage of ears with *Spm-s* activity in the indicated percentage of kernels receiving the *a-m5w* allele. The plants were grown from fully *Hfe* kernels produced on the first and second ears (left and middle panels) of plant 1466A-2. The right panel shows the comparable percentages for ears grown from kernels showing small *Hfe* sectors selected from ears of tester plants (*a sh2, wx-m8*) receiving pollen from plant 1466A-2. b, The percentage of kernels on the same ears as those for which data are given in part a, but which did not receive the *a-m5w* allele following meiotic segregation. c, The left and center panels show data comparable to those appearing in parts a and b, but for plants grown from *Hfe* kernels produced on the 1466A-2 progeny ears for which data appear in parts a and b, and which therefore constitute the subsequent generation. The right panel shows the percentage of ears showing *Spm-s* activity on plants grown from kernels selected from the same first generation progeny ears, but which did not receive the *a-m5w* allele and showed no *Spm* activity (part b, left and center panels). The ears were crossed by plants that were homozygous for the *a-m1-5719A1* allele and had an *Spm-w* element.


a.
b.
c.
% of ears
% active Spm
♀ ear 1
♀ ear 2
♂
+Spm-w
-Spm-w
1 Spm
+Spm-w reintroduced
0 20 40 60 80 100

first and second ears on the plant, as well as from kernels on tester ears that had received the *a-m5w* allele through the male gametes. Since only two *a-m5w* kernels on the tester ear showed the *Hfe* phenotype throughout the aleurone layer, kernels exhibiting small *Hfe* sectors were selected and grown to determine whether the trait was heritable, even if not expressed in kernels receiving the newly activated element through male germ cells. Plants comprising the families grown from the three groups of kernels were backcrossed to and by *dSpm* testers (see MATERIALS AND METHODS). The ears resulting from the crosses were analyzed to determine (1) whether the element was active only the presence of the *Spm-w* element and (2) whether the element was expressed in kernels that received it through male gametes. The results of the first analysis for the three families are shown in the three panels of parts a and b of Figure 3.

Hfe kernels selected from ear 1 of plant 1466A-2 gave plants that showed the *Hfe* phenotype in 50% of progeny kernels receiving the *Spm-w* element of the *a-m5w* allele (Figure 3a, left panel). None of the kernels on any of the ears produced on these plants showed *Spm* activity in the class of kernels not receiving the *Spm-w* element (Figure 3b, left panel). When plants belonging to this family were used as pollen parents, the results were consistent with those obtained in the previous generation. Only 3 of the 12 ears (25%) obtained in such tests showed any *Hfe* kernels (not shown). Those that did, had very few *Hfe* kernels (0.4, 0.6 and 2.2%). That the genetic properties of this element are quite heritable was demonstrated by selecting second-generation *Hfe* kernels and growing a third plant generation. The results obtained with ears produced on such plants (6 families, 36 plants) are displayed in the left and central panels of Figure 3c and resemble those obtained in the previous generation (Figure 3, a and b). Moreover, as in the two previous generations, the *Hfe* phenotype was observed in very few kernels on tester ears receiving pollen from such plants. *Hfe* kernels appeared on only half of the 20 tester ears receiving pollen from these plants and none of these showed more than 1.5% *Hfe* kernels (not shown).

The results obtained when *Hfe* kernels were selected from the second ear of plant 1966A-2 were comparable to those obtained with plants grown from kernels selected from the first ear (Figure 3, a and b, middle panels), except that a larger fraction of the ears on plants grown from the selected kernels had less than 20% *Hfe* kernels. None of the kernels that did not also receive the segregating *Spm-w* element showed *Spm* activity (Figure 3b, middle panel) and there were very few *Hfe* kernels on tester ears receiving pollen from these plants (not shown). Comparable results were also obtained with plants grown from kernels on tester ears that received pollen from plant 1466A-2, but which exhibited only small *Hfe* sectors (Figure 3, a and b, right panels). However, an even larger fraction (63.6%) of the ears in this family showed less than 10% *Hfe* kernels (Figure 3a, right panel). These results establish that the newly activated element in these plants, although very infrequently expressed in kernels receiving it through male germ cells, can nonetheless be transmitted through the male germline. However, they also suggest that the element reverts to the inactive form much more frequently when it is transmitted through male germ cells than when it is transmitted through female germ cells.

The observation that only half of the kernels receiving the *a-m5w* allele exhibit an *Hfe* phenotype among the progeny of plant 1466A-2 (Figure 3a, left panel) suggests the presence of an independently segregating *Modifier* element. If this is the case, then the element should also be present in half of the kernels that did not receive the *Spm-w* element of the *a-m5w* allele. To determine whether an independently segregating *Modifier* element is present, kernels that did not receive the *a-m5w* allele were selected from the same second-generation ears used for the *Hfe* selections for which the results are given in the first two panels of Figure 3c. Plants grown from these kernels were tested for the presence of a *Modifier* element by crossing them to and by plants containing an *Spm-w* element. The results obtained for the 6 families analyzed appear in the right panel of Figure 3c. Only one (1.4%) of the 67 ears obtained exhibited evidence of *Modifier* activity in more than 10% of the kernels and none showed *Modifier* activity in more than 20% of the kernels. Thus, unlike the *Modifier* element described by MCCLINTOCK (1957), the newly activated *Modifier*-like element readily reverts to a form in which it cannot be *trans*-activated upon segregation away from the active *Spm-w* element of the *a-m5w* allele.

Two cryptic elements can be activated in the same genome: Some of the plants in family 1466 and its progeny gave ears with an unexpectedly large fraction of *Hfe* kernels. A slight skewing of the distribution to higher *Hfe* frequencies is evident in the middle panel of Figure 3a and is quite pronounced in the next generation, for which comparable results are shown in the middle panel of Figure 3c. Two possible explanations for these observations are (1) that the element undergoing activation in these cultures is linked to the *a-m5w* allele and (2) that there is a second cryptic element undergoing activation in some of the plants.

Evidence that these cultures contain a second cryptic element emerged from the analysis, through two generations, of a plant grown from one of the two fully *Hfe* kernels on one of the two tester ears that

received pollen from plant 1466A-2. On the single ear of this plant (1890-1), 76.5% of the kernels that received the *a-m5-Spm-w* allele showed the *Hfe* phenotype and 47.2% of the kernels that did not receive the *a-m5w* allele gave evidence of *Spm* activity, as judged by the excision of the *dSpm* element from the *wx-m8* tester allele. These values suggested the presence of both the original *Modifier*-like element and a second, independently segregating *Spm-s* element.

To determine whether plant 1890-1 had two independently segregating elements, plants grown from *Hfe* kernels were backcrossed to and by *dSpm* tester plants. The percentages of *Hfe* kernels observed in the kernel class receiving the *a-m5w* allele and of kernels with *Spm* activity in the kernel class not receiving the *a-m5w* allele are compared for individual plants used as both egg and pollen parents in Figure 4a. Comparable data for several individual plants grown from *Hfe* kernels selected from the first ear of plant 1466A-2 are shown for comparison in Figure 4b. As described above, the activity of the *Modifier*-like element is detectable only in kernels receiving the *a-m5w* allele through the egg parent (Figure 4b).

Among the 5 plants grown from *Hfe* kernels of plant 1890-1 and for which appropriate crosses were made, one exhibited the *Modifier*-like behavior characteristic of the newly activated element in the parent plant 1466A-2 (*Mod* in Figure 4a). Two plants showed comparable fractions (40–60%) of *Hfe* kernels in the *a-m5w* class when used as either pollen or egg parent and comparably high frequencies of kernels with *Spm* activity in the class of kernels not receiving the *a-m5w* allele (*Spm* in Figure 4a). These two plants therefore appear to be segregating a single *Spm-s* element unlinked to the *a-m5w* allele. The last two plants show segregation ratios consistent with the presence of both the *Modifier*-like element and an *Spm-s* element (*Mod* and *Spm* in Figure 4a). That is, 75–80% of the kernels receiving the *a-m5w* allele through female gametes and 45–50% of those receiving it through male gametes exhibit the *Hfe* trait. In addition, half of the kernels not receiving the *a-m5w* allele show *Spm* activity. The segregation ratios observed with these two plants are the same as that observed in the previous generation (see above). Thus the *Hfe* phenotype of one of the two *Hfe* kernels obtained when plant 1466A-2 was used as a male parent is attributable to the simultaneous activation of a second, independently segregating *Spm-s* element. Moreover, the observation that there is an increasing number of plants with more than 50% *Hfe* kernels in the second generation progeny derived from plant 1466A-2 (Figure 3c, middle panel) is consistent with the progressive activation of the second cryptic element in some plants.

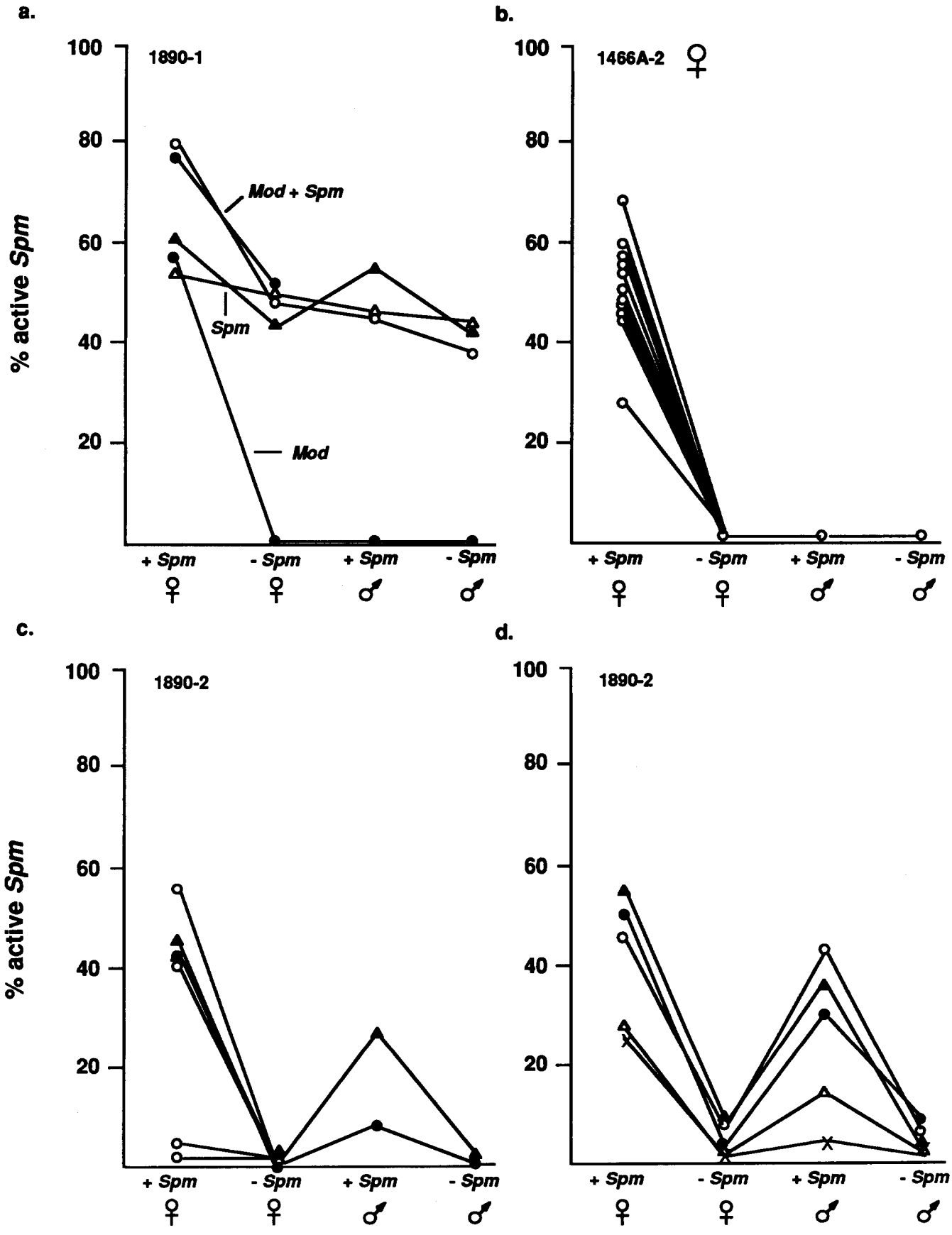
The *Modifier*-like element is an intermediate in the activation of a cryptic *Spm* element: Although the *Hfe* phenotype of the *Modifier*-like element in plant 1466A-2 and its progeny is almost never expressed in kernels receiving it through male gametes, one of the two fully *Hfe* kernels appearing on a tester ear that received pollen from plant 1466A-2 gave a plant (1890-2) that carried the *Modifier*-like element. Analysis of its progeny provided further insight into the properties of the *Modifier*-like element. Unlike any of the *Hfe* kernels that received the *Modifier*-like element from plant 1466A-2 through female gametes (Figure 3b, left and central panels), the single ear produced on plant 1980-2 had *Spm-s* activity in 4.3% of the kernels not receiving the *a-m5w* allele. Among the kernels that received the *a-m5w* allele, 33.9% exhibited the *Hfe* phenotype.

Four types of kernels were selected for further analysis from the ear produced on plant 1890-2. These were (1) kernels that showed *Spm-s* activity, but had no *Spm-w* element; (2) kernels that received the *a-m5w* allele, but had few or no *Hfe* sectors; (3) fully *Hfe* kernels in which the revertant *A* sectors were small and the *wx-m8* allele showed a late pattern of *dSpm* excision, as judged by the small size of revertant *Wx* sectors; and (4) fully *Hfe* kernels in which the revertant *A* sectors were moderately large and the *wx-m8* allele showed an early pattern of frequent *dSpm* excision, giving many *Wx* sectors, some of which were large.

Kernels that did not receive the *a-m5w* element, but showed *Spm-s* activity, were grown and tested for the presence of an *Spm-s* element. None of the six plants analyzed contained an active *Spm-s* element. Five of the six plants gave ears with no variegated kernels and the sixth had just a few such kernels (0.3%). These observations suggest that exposure to an active element can *preset* an element to be expressed after removal of the activating element, but that the *preset* element tends to return to an inactive form during development.

The six plants grown from kernels that received the *a-m5w* allele, but showed few or no *Hfe* sectors (Figure 1a) gave ears whose phenotypes were entirely consistent with earlier results. The number of kernels with *Hfe* sectors varied from 0 to 1.4%. These plants received only the *a-m5w* allele.

Plants grown from kernels exhibiting a late reversion pattern at both the *a* and *wx* loci gave a majority of plants with an element resembling the *Modifier*-like element of the parental plant (1890-2) and grandparental plant (1466A-2). In four of the six plants analyzed, 40–60% of the kernels receiving the *a-m5w* allele showed the *Hfe* phenotype, while none of the segregating kernels that did not receive the *a-m5w* allele showed *Spm* activity (Figure 4c). Two of the four plants were used as pollen parents on tester ears, and in all of the ears obtained, between 10% and 30%


a.
1890-1
100
80
60
40
20
% active Spm
Mod + Spm
Spm
Mod
+ Spm
- Spm
+ Spm
- Spm
♀
♀
♂
♂
b.
1466A-2 ♀
100
80
60
40
20
+ Spm
- Spm
+ Spm
- Spm
♀
♀
♂
♂
c.
1890-2
100
80
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40
20
% active Spm
+ Spm
- Spm
+ Spm
- Spm
♀
♀
♂
♂
d.
1890-2
100
80
60
40
20
+ Spm
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+ Spm
- Spm
♀
♀
♂
♂

of the kernels that received the *a-m5w* allele showed the *Hfe* phenotype. Segregating kernels on the same ears that did not receive the *a-m5w* allele showed no *Spm* activity. These observations suggest that the *Modifier*-like element in these two plants has been altered in a way that increased its ability to be expressed in kernels that received it through male germ cells. Finally, two of the six plants analyzed showed less than 10% *Hfe* kernels among those that received the *a-m5w* allele. This is consistent with the observation that the *Modifier*-like element is less often transmitted in an active form through male than female germ cells from the original plant (1466A-2, Figure 3a, right panel).

Seven plants were grown from *Hfe* kernels showing an early reversion pattern at both *a* and *wx* loci. Similar results were obtained with all of them and data for five of the seven plants are shown in Figure 4d. Among the plants selected for an early reversion pattern, expression of the *Hfe* phenotype in kernels that received the *Modifier*-like element through male germ cells is further enhanced. Three of the six plants that were used as a pollen parent gave ears on which 30–44% of the *a-m5w* kernels showed the *Hfe* phenotype. Moreover, there was a marked increase in the fraction of segregating kernels (no *a-m5w*) that showed *Spm-s* activity. These observations suggest that although the genetic behavior of the *Modifier*-like element originally identified in plant 1466A-2 is relatively stable, it is also subject to further changes that increase its resemblance to a standard *Spm* element. The genetic change that permitted its expression in a kernel that received it through a male gamete probably occurred late in the development of plant 1466A-2. The alteration was transmitted through the ear parent in the next generation (plant 1890-2) and was reflected in the increased expression of the *Hfe* phenotype received through the male germline in the following generation (Figure 4, c and d). The most striking property of the *Modifier*-like element is that its expression is dependent on the presence of an activating *Spm* element. Hence, as has been observed in other studies, the *Spm* element can exist in a heritable, metastable form in which its expression is inducible by a gene product encoded by the element itself (McClintock 1958, 1971; Banks, Masson and Fedoroff 1988).

Cryptic elements can be heritably reactivated: In order to determine whether newly activated elements remain subject to inactivation when the *trans*-activating element is removed or whether they become stably active, selections were made from ears in which the segregation ratios were those expected for a fully active, independently segregating *Spm-s* element. The families within which the selections were made arose from three plants: 1466A-1, 1473A-1, and 1466B-4. The first two were grown from fully *Hfe* kernels appearing on parental ears carrying the *a-m5w* allele and themselves showing a low frequency of *Hfe* kernels and kernel sectors. Plant 1466B-4 was grown from a kernel selected from the same ear as that giving rise to plant 1466A-1, but which had a large *Hfe* sector in the kernel aleurone layer. Progeny ears from test crosses to and by plants 1466A-1 and 1473A-1 gave evidence of the presence of a fully active *Spm-s* element segregating independently of the *a-m5w* allele. That is, half of the kernels that received the *a-m5w* allele showed the *Hfe* phenotype and half of the kernels that did not receive the *a-m5w* allele showed *Spm-s* activity, as judged by excision of the *dSpm* element of the *wx-m8* allele. Plant 1466B-4 was used only as a male parent and gave 0.3–1.6% *Hfe* kernels in *a-m5w* class in the several test ears that received pollen from the plant.

Fourteen progeny plants were grown from *Hfe* kernels selected from ears on plants 1466A-1 and 1473A-1 and all of them showed the segregation ratios expected for the presence of an independently segregating *Spm-s*. About half (44.7–55.9%) of the *a-m5w* class of kernels exhibited the *Hfe* phenotype and about half (40.0–57.9%) of the kernels that did not receive the *a-m5w* allele showed *Spm-s* activity. Two of the three plants grown from the three *Hfe* kernels on one of the ears that received pollen from plant 1466B-4 gave *Hfe* and *Spm-s* segregation ratios consistent with the presence of a fully active, independently segregating *Spm-s* element, while the third gave no evidence of an *Spm-s* element. Kernels that did not receive the *a-m5w* allele, but did receive an active *Spm-s* element, as judged by the reversion of the *wx-m8* allele in kernel

Figure 4.—Progeny analysis of plants grown from *Hfe* kernels appearing on a tester ear receiving pollen from plant 1466A-2. Ears produced by plants 1890-1 and 1890-2, grown from 2 *Hfe* kernels on a tester ear that received pollen from plant 1466A-2, were the source of the *Hfe* kernels selected for growth and subsequent analysis (parts a, c and d). a, The results obtained from progeny of plant 1890-1. b, The results of comparable progeny testing on plants grown from *Hfe* kernels produced on the parental plant 1466A-2. c, The results of progeny testing on plants grown from *Hfe* kernels produced on plant 1890-2 and which exhibited late, frequent excision of the *Spm-w* and *dSpm* insertions at the *a* and *wx* loci, respectively; d, The results of progeny testing on *Hfe* kernels selected from the ear produced by plant 1890-2, but which showed early and frequent reversion at both *a* and *wx* loci. Each line connects the percentages of kernels with *Spm-s* activity on the first ear of a given plant (female transmission, ♀) and on a tester that received its pollen (male transmission, ♂). The first value in each pair is the percentage of *Hfe* and *Hfe*-sectored kernels (percent active *Spm*) in the class receiving the *a-m5w* allele (+*Spm*) and the second value is the percentage of kernels exhibiting *Spm* activity, as judged by the ability to excise the *dSpm* of a tester allele, in the kernel class that did not receive the *a-m5w* allele following meiotic segregation (−*Spm*). All of the plants were *a-m5w/a* heterozygotes.

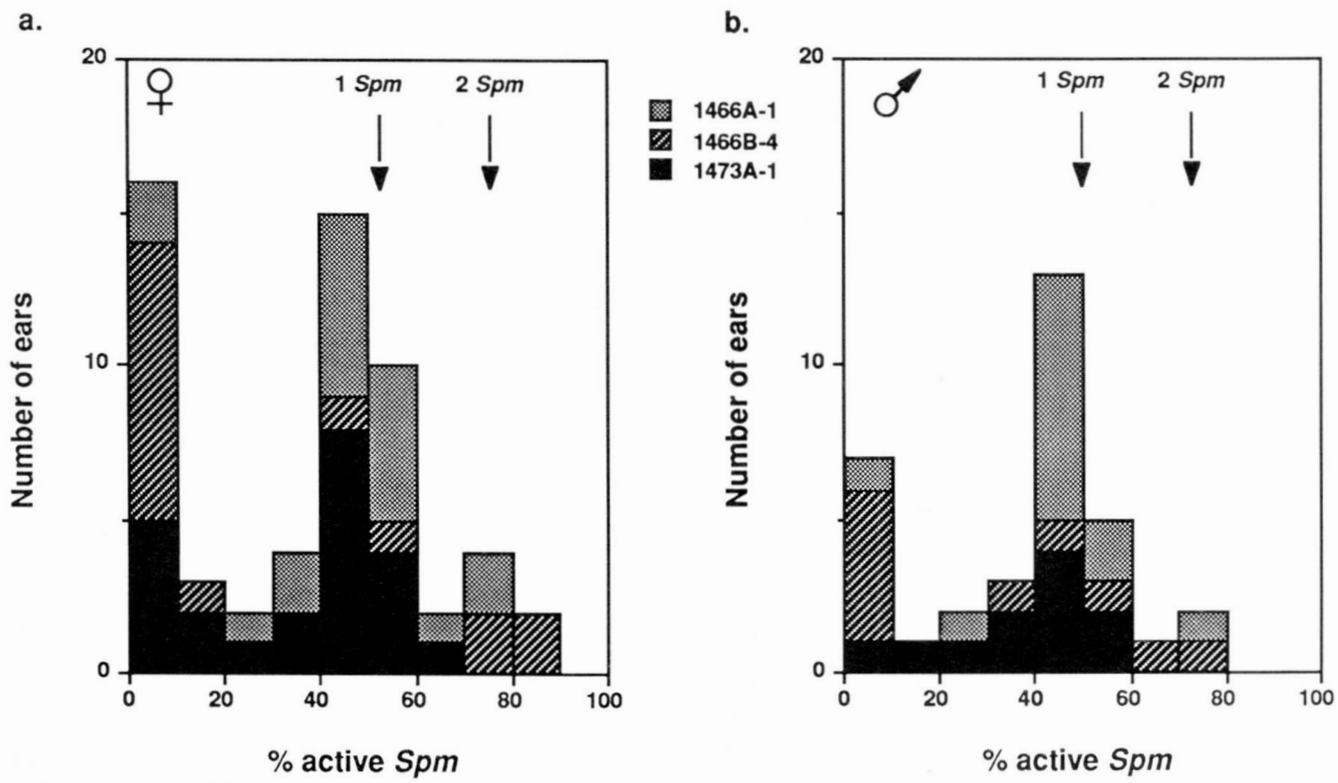

FIGURE 5.—The ability of activated *cryptic Spm-s* elements to remain in a heritably active form. All of the kernels were selected from ears which gave evidence of a fully active *Spm-s* element segregating independently of the *a-m5w* allele. As described in the text, the tested progeny of plant 1473A-1 and 1466A-1 represented the second generation exhibiting a fully active *Spm-s*, while the tested progeny of 1466B-4 belonged to the first generation with a fully active *Spm-s* element. Plants were grown from kernels which received the newly activated *cryptic Spm* element, but not the *a-m5w* allele. All of the plants were homozygous for the *a* and *wx-m8* alleles and the presence of the activated *cryptic Spm* was judged by the excision of the *dSpm* element of the *wx-m8* allele. All plants were used as female (panel a) and male (panel b) parents in crosses to and by testers that were homozygous for both the *a-m1-5719A1* and *wx-m8* tester alleles and the resulting ears were scored for the percentage of kernels exhibiting *Spm-s* activity, as judged by the excision of the *dSpm* element of the tester allele. The number of ears in each family descended from the indicated grandparental plant (1966A-1, 1966B-4, 1973A-1) exhibiting the indicated percentages of kernels with an active *Spm-s* element is represented by a different shading pattern.

endosperm tissue, were subsequently selected from the ears produced by the second generation of plants and grown to determine whether the *Spm-s* had been heritably activated. It should be emphasized that in two of the three lineages, those derived from plants 1466A-1 and 1473A-1, a newly activated, independently segregating element had been present for two generations, while in the third lineage, derived from plant 1466B-4, the ears from which kernels were selected were the first ones to give evidence of a fully active *Spm-s* element.

Plants grown from the selected kernels exhibiting *Spm-s* activity were crossed by and to testers that were homozygous for the *a-m1-5719A1* and *wx-m8 dSpm* alleles. The resulting ears were scored for the percentage of kernels exhibiting *Spm-s* activity. The data obtained for the families descended from the three grandparental plants are represented by different shading patterns in the histograms shown in Figure 5, a and b. The distributions obtained when the test plants were used as either pollen or egg parents are trimodal, with peaks at 0, 50 and 75% *Spm-s*-containing kernels per ear (Figure 5). The peak at 75% *Spm-s*-containing kernels per ear consists only of ears resulting from crosses involving plants descended from family 1466. Ears containing two independently segregating *Spm-s* elements would be expected to show variegation in 75% of the kernels. Evidence has already been presented, based on the analysis of the progeny of plant 1466A-2, that there are two independently segregating cryptic elements returning to an active state in this family (Figure 4).

Plants belonging to the same family, but differing by the number of generations since full reactivation of the cryptic element differed markedly in the heritability of the newly activated element (Figure 5). In the progeny of plant 1466A-1, 10.5 and 7.6% of the ears receiving the newly activated element through female and male gametes, respectively, showed *Spm-s* activity in less than 20% of the kernels. By contrast, 62.5 and 50%, respectively, of ears receiving the newly activated element from progeny of plant 1466B-4 through female and male gametes showed *Spm-s* activity in less than 20% of the kernels. The

corresponding percentages were 30.4 and 9.1 for the families derived from plant 1473A-1. It appears, therefore, that the tendency of a newly activated element to return to an inactive state decreases when it is maintained in the presence of the *trans*-activating *Spm-w* element for a second generation. Finally, it should be noted that the peaks at 0 and 50% *Spm-s*-containing kernels are well separated. The marked clustering of values suggests that a newly activated element either rapidly returns to a genetically silent state upon segregation from the *trans*-activating element or achieves a stably and heritably active state. Since all of the plants were grown from kernels exhibiting *Spm* activity, this observation lends further weight to the inference that the *trans*-acting *Spm* element can *preset* a *cryptic* element to be expressed following removal of the active element without assuring its heritable reactivation.

DISCUSSION

Spm elements can exist in three different forms: The picture of the *Spm* element that emerges from these and other recent studies is that it can exist in one of many states belonging to a continuum that can be subdivided into three major categories (FEDOROFF and BANKS 1988; BANKS, MASSON and FEDOROFF 1988). The first of these categories comprises the *cryptic* states, in which the element is neither expressed nor can it be transcriptionally *trans*-activated by an active element. The second category, designated *programmable*, comprises elements in a variety of unstable states that show different programs of element expression during plant development. It is characteristic of inactive *Spm* elements in the *programmable* form that they are readily *trans*-activated by another *Spm* element in the same genome, which also promotes their conversion to a heritably active form at a high frequency (BANKS, MASSON and FEDOROFF 1988). Finally, elements can exist in a stably active form, which is itself highly heritable and in which the element is subject to inactivation very infrequently (FEDOROFF and BANKS 1988).

Multiple states can be identified within the *cryptic* category: The results of the present study show that the *Spm* element can exist in multiple, genetically distinguishable states within the *cryptic* category. *Cryptic Spm* elements differ in their tendency to undergo spontaneous reactivation. This is evidenced by clustering of spontaneous reactivation events in plant families. It is not clear from the existing data whether the variation is attributable to a stochastic component within the mechanism that maintains the element in a genetically silent form or whether it is attributable to genetic variables that have not yet been identified and analyzed.

A *trans*-activating *Spm* element alters the heritability of the cryptic state: The presence of an active *Spm* element increases the probability that a cryptic element will be activated. Extensive evidence has been presented to support the thesis that *Hfe* sectoring on kernels carrying an *Spm-w* element is symptomatic of the *trans*-activation process. Selection of kernels giving evidence of sectorial element reactivation efficiently selects for plants with activated *cryptic Spm* elements. Although the newly activated cryptic elements tend to return to an inactive form when segregated away from the activating element in the first generation after activation, the heritability of the active state increases when the newly activated element remains in the presence of the *trans*-activating element for a second generation.

When a *trans*-activating *Spm* element is removed from a genome containing a *cryptic Spm* element, it becomes apparent that the cryptic element has undergone a heritable alteration in its ability to undergo spontaneous activation. The spontaneous activation frequency is about two orders of magnitude higher immediately after segregation of a *trans*-activating element than it is in a baseline population of plants used here and propagated for several generations without an active *Spm* element. One generation after the removal of the *trans*-activating element, the probability of spontaneous activation is roughly tenfold lower, but remains about an order of magnitude higher than the baseline probability of spontaneous activation in the present genetic background. It follows that exposure to a *trans*-acting *Spm* element causes a heritable genetic change in a cryptic element that predisposes it to spontaneous activation. While heritable, the change is not permanent and appears to be reversed in the following generations, decreasing the frequency of spontaneous activation.

The *Modifier*-like element is a new element belonging to the *programmable* category: Evidence has been presented that early in the activation of a cryptic element, the element goes through an intermediate stage in which it is active only in the presence of the *trans*-activating element and readily reverts to a cryptic state. An element in this form was identified and shown to be heritably maintained in this form through three plant generations. The element was designated *Modifier*-like because it differs in two respects from the *Modifier* element belonging to the *Spm* family identified by MCCLINTOCK (1957).

MCCLINTOCK's *Modifier* element is active only in the presence of a *trans*-activating element, a property shared by the *Modifier*-like element of the present study. However, the present *Modifier*-like element, unlike MCCLINTOCK's *Modifier*, is almost never expressed in kernels receiving it through the pollen parent, is poorly transmitted through the male germline, and returns to a genetically undetectable form

immediately upon segregation from the *trans*-activating element. Moreover, although the properties of the *Modifier*-like element are heritable and stable when the element is propagated through the egg parent, selection for expression through the pollen parent revealed it to be subject to further modifications. Selection among the progeny of such a plant resulted in a marked increase in the ability of the *Modifier*-like element to be active in kernels receiving it through the pollen parent, as well as in the absence of the *trans*-activating element. It appears likely, therefore, that the *Modifier*-like element represents a *cryptic Spm* element in a genetically metastable, partially activated form.

Efforts to derive a fully active *Spm* element from a *Modifier* element received from McCLINTOCK have been unsuccessful despite considerable effort (N. FEDOROFF, unpublished data). It is possible, however, that the same genetic form of the element can result from either an alteration in the element's nucleotide sequence or from a reversible epigenetic modification. It has already been noted that similar *Spm* element-associated mutant phenotypes can result from intra-element deletions and from extensive methylation of element sequences (BANKS, MASSON and FEDOROFF 1988).

The *Modifier*-like element represents a new member of the category of elements termed *programmable*. The element exhibits a highly reproducible differential pattern of expression in the plant and is readily *trans*-activated in the presence of an active element. Moreover, it is capable of further modifications which can either return it to a cryptic form or promote its conversion to a fully active *Spm* element. As with other *programmable* forms of the element that have been studied, the *Modifier*-like element has a higher tendency to return to a cryptic form upon transmission through the male than through the female germline (FEDOROFF and BANKS 1988; BANKS, MASSON and FEDOROFF 1988).

How does the *Spm* element promote reactivation of *cryptic Spm* elements? It was recently reported that the *cryptic*, inactive *programmable*, and active (stable or *programmable*) forms of the *Spm* element differ markedly in the extent of methylation of C residues in a region of the element immediately around its transcription start site, located 0.2 kb from the element's 13-bp terminal inverted repeat (BANKS, MASSON and FEDOROFF 1988). Changes in element sequence methylation correlated with the element's state of activity were confined to the 0.2-kb sequence upstream of its transcription start site (termed the upstream control region or UCR) and the 0.35-kb, GC-rich sequence immediately downstream of its transcription start site and contained within its first, untranslated exon (termed the downstream control region or DCR). The remainder of the element sequence was found to be methylated at all of the sites examined regardless of its state of activity.

Transcriptionally active *Spm* elements are distinguishable from transcriptionally silent elements by the extent of methylation at certain sites within the UCR. Active, inactive *programmable*, and *cryptic* elements are virtually unmethylated, partially methylated and almost fully methylated, respectively, at certain sites within the DCR. Upon exposure to a *trans*-activating *Spm* element, *programmable* inactive elements are transcriptionally activated and methylation is no longer detectable at the several sites examined in both the UCR and DCR sequences (BANKS, MASSON and FEDOROFF 1988). Inactive *programmable Spm* elements also show a marked increase in the heritable reactivation of the inactive *Spm* element after segregation of the *trans*-activating element.

The heritable changes in *cryptic Spm* elements investigated in the present study are also probably attributable to changes in the extent and pattern of methylation within the element's UCR and DCR sequences. *Cryptic Spm* elements, although not subject to transcriptional *trans*-activation, show reduced methylation at sites within the UCR and DCR in the presence of a *trans*-activating *Spm-w* element (BANKS, MASSON and FEDOROFF 1988). The ability of the *trans*-acting element to increase the probability of spontaneous activation of a *cryptic Spm* may be attributable to the ability of the element-encoded autoregulatory gene product to promote a reduction in methylation of the UCR and DCR. Such an explanation is consistent with the transient heritability of the *trans*-acting element's influence. Thus, the partial demethylation promoted by the element-encoded gene product may not suffice to reactivate the element in a single generation, but may make it more likely that an element undergoes spontaneous reactivation simply by reducing the overall methylation level. The persistence of the effect through at least one generation further suggests that element remethylation is a gradual process, an observation that is consistent with previous observations on the gradual increase over several generations in both the stability of the inactive state and the methylation level within the UCR and DCR of a newly inactivated element (FEDOROFF and BANKS 1988; J. A. BANKS, unpublished observations).

The results of the present genetic studies suggest that the *trans*-activating element must be present through several plant generations to promote the complete conversion of an element from a *cryptic* to a stably active form. If, as appears likely, element activation is effected by reducing methylation of the element's UCR and DCR sequences, then it follows that the demethylation process requires several plant generations and proceeds through genetically meta-

stable, partially methylated intermediates in which the element's promoter is inducible in the presence of an element-encoded gene product (BANKS, MASSON and FEDOROFF 1988).

A *trans*-activating *Spm-w* can preset a *cryptic Spm* for transient expression: The ability of the *a* gene to be *preset* for subsequent expression is a property of certain *dSpm* derivatives of MCCLINTOCK's original *a-m2* allele, in which an *Spm* element inserted just upstream of the *a* gene, bringing the gene's expression under the control of the element's regulatory system (MCCLINTOCK 1962; MASSON *et al.* 1987). *Presetting* of the *a* gene by a *trans*-acting *Spm* element is mediated by sequences near the ends of the *dSpm* element. It has been proposed that *presetting* is attributable to an interaction between an element-encoded positive autoregulatory gene product and element sequences that prevents heritable modifications that inactivate element expression (MASSON *et al.* 1987). This proposal finds support in the present observations that a *trans*-acting *Spm-w* element can *preset* a *cryptic Spm* for transient expression after the element is removed by segregation. That is, we have analyzed *cryptic Spm* elements that are sufficiently activated to be expressed in kernels receiving them and not the *trans*-activating element, but which nonetheless show little or no evidence of heritable activation.

New developmental programs of *Spm* expression: We have identified two new types of *programmable Spm* elements. One of these resembles MCCLINTOCK's *Modifier* element, but is expressed almost exclusively in kernels receiving it through the egg parent. The second type of element is one that can be *preset* by a *trans*-activating element to be expressed in kernels that receive it, but not the *trans*-activating element, yet is not heritably activated. It is likely that at least some of these metastable genetic programs are attributable to specific patterns of methylation within the element's UCR and DCR. For example, there is evidence that the methylation state of the UCR determines whether or not the element is transcribed, while the methylation state of the DCR governs the heritability of the element's activity phase (BANKS, MASSON and FEDOROFF 1988). Thus a *preset* element may be one in which the UCR is hypomethylated, while the DCR is extensively methylated, permitting its transient expression until the UCR is remethylated.

Perhaps the most important insight gained from this and other studies on the regulation of the *Spm* element is that the *Spm* element's developmental expression pattern is determined by an interactive genetic system comprising four components (1) the nucleotide sequence of the *Spm* element, (2) the methylation pattern of sequence around the element's transcription start site, (3) the presence of an element-encoded autoregulatory gene product and (4) an inactivation system that is differentially expressed or imposed during plant development (FEDOROFF and BANKS 1988; BANKS, MASSON and FEDOROFF 1988). The rapidity with which different developmental programs of *Spm* expression can be isolated by selection implies that a given epigenetic setting is not static, but constantly changing. The results of both the present and earlier studies indicate that an element's program can be altered during development (FEDOROFF and BANKS 1988). Indeed, the very regular pattern of change in element expression during development implies that the pattern itself results from "erasure" and reimposition of element-inactivating modifications as development progresses. How the element responds depends on its initial epigenetic state. That state can, in turn, be modified both by the plant-imposed inactivation system and the element's own autoregulatory gene product.

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